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(54) Title: VACCINE FOR <i>BRANHAMELLA CATARRHALIS</i>		
(57) Abstract Compositions comprising outer membrane protein "CD", and peptides and oligopeptides thereof, of <i>Branhamella catarrhalis</i> are described. Additionally, nucleotide sequences encoding the protein, peptide or oligopeptide are disclosed, as well as recombinant vectors containing these sequences. Protein, peptide or oligopeptide can be produced from host cell systems containing these recombinant vectors. Peptides and oligopeptides can also be chemically synthesized. Disclosed are the uses of the protein, peptides and oligopeptides as antigens for vaccine formulations, and as antigens in diagnostic immunoassays. The nucleotide sequences are useful for constructing vectors for use as vaccines for insertion into attenuated bacteria in constructing a recombinant bacterial vaccine, and for inserting into a viral vector in constructing a recombinant viral vaccine. Also described is the use of nucleotide sequences related to the gene encoding CD as primers and/or probes in molecular diagnostic assays for the detection of <i>B. catarrhalis</i> .		

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VACCINE FOR *BRANHAMELLA CATARRHALIS*

This invention was made with government support under grant A128304 awarded by the National Institutes of Health. The government has certain rights in the invention.

5 This application is a continuation-in-part of my earlier co-pending application United States Serial No. 08/129,719, filed September 29, 1993, which is incorporated herein by reference.

Field of Invention

10 The present invention relates to compositions comprising a protein, and peptides and oligopeptides thereof, associated with the outer membrane of *Branhamella catarrhalis*. More particularly, the invention is directed to compositions of a protein, and
15 peptides and oligopeptides thereof, related to an outer membrane protein, "CD", of apparent molecular mass of about 55,000 to 60,000 daltons found in *B. catarrhalis*. Also disclosed is methods for preparing CD and CD
20 peptides using recombinant DNA and/or biochemical techniques. Related thereto, disclosed is the DNA sequence encoding CD, and recombinant vectors useful in directing the expression of CD and CD peptides and oligopeptides, and host cells transformed with such recombinant vectors.

25 The proteins, peptides, and oligopeptides are used as immunogens in vaccine formulations for active immunization; and can be used to generate protein-specific and peptide-specific antisera useful for passive immunization, and as reagents for diagnostic
30 assays. The nucleotide sequences disclosed provide for the synthesis of corresponding oligonucleotides which can be used as reagents in diagnostic assays directed to the detection of *B. catarrhalis* genetic material, and incorporated into expression vectors for use as genetic
35 vaccine formulations.

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Background of the Invention

Branhamella catarrhalis (also known as *Moraxella catarrhalis*) is an important human respiratory tract pathogen. *B. catarrhalis* is the third most common cause of otitis media in infants and children, after *Streptococcus pneumoniae* and nontypeable *Haemophilus influenzae*, as documented in studies in which tympanocentesis has been used to establish the etiologic agent (Murphy, 1989, *Pediatr. Infect. Dis. J.* 8:S75-S77). *B. catarrhalis* is a common cause of sinusitis and conjunctivitis in both children and adults (See for example, Bluestone, 1986, *Drugs* 31:S132-S141; Brorson et al., 1976, *Scand. J. Infect. Dis.* 8:151-155; and Romberger et al., 1987, *South. Med. J.* 80:926-928); and is an important cause of lower respiratory tract infections in adults with chronic bronchitis and chronic obstructive pulmonary disease (Murphy et al., 1992, *Am. Rev. Respir. Dis.* 146:1067-1083; Catlin, 1990, *Clin. Microbiol. Rev.* 3:293-320). Additionally, *B. catarrhalis* can cause pneumonia, endocarditis, septicemia, and meningitis in immunocompromised hosts (Cocchi et al., 1968, *Acta Paediatr. Scand.* 57:451-3; Douer et al., 1977, *Ann. Intern. Med.* 86:116-119; McNeely et al., 1976, *Am. Rev. Respir. Dis.* 114:399-402).

Since recurrent otitis media is associated with substantial morbidity, there is interest in identifying strategies for preventing these infections. One such approach is the development of vaccines. An effective vaccine for preventing bacterial otitis media would need to include antigens which would generate protection against infection by *S. pneumoniae*, nontypeable *H. influenzae* and *B. catarrhalis*. Indeed, vaccine development for the pneumococcus and nontypeable *H. influenzae* are progressing such that potentially protective antigens have been identified and are currently undergoing testing (See for example, Murphy et

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al., U.S. Patent No. 5,173,294; and Vella et al., 1992, *Infect. Immun.* 60:4977-4983). As these vaccines are developed and used more widely, the relative importance of *B. catarrhalis* as a cause of otitis media will
5 increase in the next decade. Besides infants and children benefitting from a vaccine to prevent otitis media caused by *B. catarrhalis*, adults with chronic obstructive pulmonary disease, and immunocompromised children and adults would benefit from a vaccine to
10 prevent infections caused by *B. catarrhalis*.

Bacterial components which have been investigated as potential vaccine antigens include polysaccharides, lipopolysaccharides or modifications thereof, and outer membrane proteins. In general, as exemplified by the
15 type b capsular polysaccharide of *H. influenzae*, polysaccharide antigens have been shown to be a poor immunogen in children under the age of 18 months. Active immunization with lipopolysaccharide (LPS) is unacceptable due to its inherent toxicity. The
20 pathophysiologic effects of LPS may include fever, leucopenia, leucocytosis, the Schwartzman reaction, disseminated intravascular coagulation, and in large doses, shock and death. In general, proteins are immunogenic in infants around three months of age. Thus,
25 outer membrane proteins are being investigated as possible vaccine antigens.

While recent studies have begun to focus on outer membrane proteins of *B. catarrhalis*, little is known about the antigenic and molecular structure of these
30 proteins. Studies of purified outer membranes by SDS-PAGE have revealed a rather homogeneous pattern among strains of the bacterium (Bartos and Murphy, 1988, *J. Infect. Dis.* 158:761-765). Eight major outer membrane proteins, designated by the letters A-H, have
35 been identified (Murphy et al., 1989, *Microbial Pathogen.* 6:159-174; Bartos et al., 1988, *J. Infect.*

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Dis. 158: 761-765). Outer membrane proteins C and D differ slightly in apparent molecular mass, and thus appear as a doublet on SDS-PAGE electrophoresis. Monoclonal antibodies have been developed to *B. catarrhalis* resulting in two monoclonal antibodies, 7D6 and 5E8, which recognized both proteins C and D (Sarwar et al., 1992, *Infect. Immun.* 60:804-809). Prior to the development of the present invention, it was unknown whether this doublet represented a single protein (CD) with two stable conformations, or whether C and D are two closely related proteins encoded by different genes (Sarwar et al., *supra*). Proteins C and D are of interest, particularly for vaccine development, because these proteins express at least one conserved epitope on the surface of intact *B. catarrhalis* (Sarwar et al., 1992, *supra*).

Hence, with the increasing recognition of *B. catarrhalis* as an important bacterial pathogen, there is a need for a vaccine that is immunogenic in children and adults. Such a vaccine would have to be directed to a bacterial component which has a surface-exposed epitope on intact bacteria, wherein the epitope is conserved amongst strains of *B. catarrhalis*.

25 Summary of the Invention

The present invention is directed to a protein and peptides related to an outer membrane protein having an apparent molecular mass of about 55,000 to 60,000 daltons of *B. catarrhalis*, wherein the protein was formerly thought to be two related proteins, C and D, but which through recombinant DNA techniques disclosed herein, is now known to be one protein, CD, which is heat modifiable resulting in the appearance of two proteins differing by migration in SDS gels. The CD protein, and peptides (herein termed "CD peptides") and oligopeptides (herein termed "CD oligopeptides")

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thereof, of the present invention may be used as immunogens in prophylactic and/or therapeutic vaccine formulations; or as an antigen in diagnostic immunoassays directed to detection of *B. catarrhalis* infection by measuring an increase in serum titer of *B. catarrhalis*-specific antibody. Also, CD protein, CD peptides, and CD oligopeptides of the present invention may be used to generate CD-specific antibody which may be useful for passive immunization and as reagents for diagnostic assays directed to detecting the presence of *B. catarrhalis* in clinical specimens. CD peptides or CD oligopeptides can be obtained by chemical synthesis, purification from *B. catarrhalis*, or produced from recombinant vector expression systems using the nucleic acid sequences disclosed herein.

One embodiment of the present invention is directed to the construction of novel DNA sequences and vectors including plasmid DNA, and viral DNA such as human viruses, animal viruses, insect viruses, or bacteriophages which can be used to direct the expression of CD protein, CD peptides, or CD oligopeptides in appropriate host cells from which the expressed protein or peptides may be purified.

Another embodiment of the present invention also provides methods for molecular cloning of the gene encoding CD, and gene fragments encoding CD peptides or CD oligopeptides. The nucleic acid sequences of the present invention can be used in molecular diagnostic assays for *B. catarrhalis* genetic material through nucleic acid hybridization, and including the synthesis of CD sequence-specific oligonucleotides for use as primers and/or probes in amplifying, and detecting amplified, nucleic acids.

Additionally, CD protein, CD peptides, and CD oligopeptides can be used as immunogens in prophylactic and/or therapeutic vaccine formulations against

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pathogenic strains of *B. catarrhalis*, whether the immunogen is chemically synthesized, purified from *B. catarrhalis*, or purified from a recombinant expression vector system. Alternatively, the gene encoding CD, or one or more gene fragments encoding CD peptides or CD oligopeptides, may be incorporated into a bacterial or viral vaccine comprising recombinant bacteria or virus which is engineered to produce one or more immunogenic epitopes of CD by itself, or in combination with immunogenic epitopes of other pathogenic microorganisms. In addition, the gene encoding CD or one or more gene fragments encoding CD peptides or CD oligopeptides, operatively linked to one or more regulatory elements, can be introduced directly into humans to express protein CD, CD peptide, or CD oligopeptides to elicit a protective immune response.

Brief Description of the Figures

FIG. 1 represents a map of plasmid pCD-1, constructed from pET11b and a 2.4 kb fragment containing the gene which encodes CD. The shaded region represents the DNA insert and the thicker shaded region represents the CD gene. Abbreviations used are as follows: Ap: ampicillin resistance coding region; Lac: lac operon; bp: base pairs.

FIG. 2 represents a map of plasmid pCD-2, constructed from pGEM7zf- and a 1.5 kb fragment containing the gene which encodes CD. The shaded region represents the DNA insert and the thicker shaded region represents the gene encoding CD. Abbreviations used are as follows: Ap: ampicillin resistance coding region; Lac: lac operon; bp: base pairs.

FIG. 3 represents an immunoblot assay with whole cell lysates of: lane a: *E. coli* HB101 transformed with

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pGEM7zf-; lane b: *E. coli* HB101 transformed with pCD-2 which contains the gene encoding CD; and lanes c and d: *B. catarrhalis* strain 25240. Lanes b and c contain similar amounts of protein (~20 μ g) while, lane d contains less protein to show the characteristic doublet of CD. Samples were incubated at 100°C for 10 minutes in sample buffer containing 0.06 M Tris, 1.2% sodium dodecyl sulfate, 12% glycerol, and 5.8% β -mercaptoethanol. The immunoblot was developed with antibody 5E8 which recognizes an epitope on the CD protein. Molecular mass markers are noted on the left in thousands of daltons.

FIG. 4 represents a Southern blot assay in which purified genomic DNA of 13 strains of *B. catarrhalis* was cut with EcoRI and probed with two labeled oligonucleotides corresponding to CD gene sequence. The arrow denoting the 3.7 kilobase band represents a fragment upstream of the EcoRI site within the gene encoding CD and the arrow denoting the 325 base pair band represents the fragment downstream of the EcoRI site. Lanes contain DNA from the following strains from left to right: 105, 112, 135, 3, 6, 10, 20, 27, 31, 40, 42, 45, 56.

FIG. 5 represents a Southern blot assay in which purified genomic DNA of 14 strains of *B. catarrhalis* was cut with EcoRI and PstI and probed with a labeled oligonucleotide corresponding to sequence between the EcoRI and PstI sites within the gene encoding CD. Lanes contain DNA from the following strains from left to right: 555, 556, 585, 3583, 3614, 4223, 4629, 5193, 6951, 9928, 25240, M3, M3, M9.

FIG. 6 represents a tricine-sodium dodecyl sulfate gel stained with Coomassie blue. Lane A: purified CD.

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Lane B: Purified CD cleaved with cyanogen bromide. The arrows denote the fragments resulting from cyanogen bromide cleavage (calculated sizes of these fragments appear in Table 2). The double band below the large
5 fragment in lane B is a result of nonspecific proteolytic breakdown of the protein which is frequently observed with CD. Molecular mass markers are noted on the right in thousands of daltons.

10 Detailed Description of the Invention

The present invention is directed to compositions of a bacterial outer membrane protein, and peptides thereof, of *B. catarrhalis* wherein the protein has been designated "CD". Using SDS-PAGE, the CD protein migrates
15 as a doublet of two bands, characteristic of a heat-modifiable protein, with an apparent molecular mass of about 55,000 to 60,000 daltons. As indicated by one nucleotide sequence of the present invention (SEQ ID NO.14), the gene encoding CD reveals that the predicted
20 amino acid sequence of the mature CD protein has a calculated molecular mass of about 45,788 daltons. The CD protein, CD peptides, and CD oligopeptides of the present invention can be produced using recombinant DNA methods as illustrated herein, or can be synthesized
25 chemically from the amino acid sequence disclosed in the present invention. Additionally, peptides can be produced from enzymatic or chemical cleavage of the mature protein. CD protein, CD peptides, and CD oligopeptides with an immunogenic epitope(s), can be
30 used as immunogens in various vaccine formulations in the prevention of otitis media, sinusitis, conjunctivitis, and lower respiratory tract infections caused by *B. catarrhalis*. Additionally, according to the present invention, the CD protein and CD peptides
35 produced may be used to generate *B. catarrhalis*-specific

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antisera useful for passive immunization against infections caused by *B. catarrhalis*.

The present invention further provides the nucleotide sequence of the gene encoding CD, as well as
5 the amino acid sequence deduced from the isolated gene. According to one embodiment of the present invention, using recombinant DNA techniques the gene encoding CD, or gene fragments encoding one or more CD peptides having an immunogenic epitope(s), is incorporated into
10 an expression vector, and the recombinant vector is introduced into an appropriate host cell thereby directing the expression of these sequences in that particular host cell. The expression system, comprising the recombinant vector introduced into the host cell,
15 can be used (a) to produce CD protein, CD peptides and CD oligopeptides which can be purified for use as an immunogen in vaccine formulations; (b) to produce CD protein, CD peptides, and CD oligopeptides to be used as an antigen for diagnostic immunoassays or for generating
20 *B. catarrhalis*-specific antisera of therapeutic and/or diagnostic value; c) or if the recombinant expression vector is a live virus such as vaccinia virus, the vector itself may be used as a live or inactivated vaccine preparation to be introduced into the host's
25 cells for expression of CD or immunogenic CD peptides or CD oligopeptides; d) for introduction into live attenuated bacterial cells which are used to express CD protein, CD peptides or CD oligopeptides to vaccinate individuals; e) or for introduction directly into an
30 individual to immunize against the encoded and expressed CD protein, CD peptide, or CD oligopeptide.

For purposes of the description, the methods and compounds of the present invention will be illustrated in the following embodiments:

35 Embodiment A- Molecular cloning and sequencing of the gene encoding CD, and vectors expressing CD epitopes;

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- Embodiment B- Conservation of the gene encoding CD amongst *B. catarrhalis* strains;
- Embodiment C- Methods for using CD-specific nucleotide sequences in molecular diagnostic assays for the
- 5 detection of *B. catarrhalis*;
- Embodiment D- Characterization of CD including generation of CD peptides;
- Embodiment E- Methods for using CD, or CD peptides, in diagnostic immunoassays;
- 10 Embodiment F- Methods and compounds for vaccine formulations related to CD and CD peptides.

Embodiment A

- Molecular cloning and sequencing of the gene encoding
- 15 CD, and vectors expressing CD epitopes.

- The strategy used was to isolate genomic DNA from *B. catarrhalis*, cleave the isolated DNA into fragments, construct a genomic library comprising insertion of the fragments into an expression vector, introduce the
- 20 recombinant vectors into the appropriate host cell, and immunoscreen for host cell clones expressing CD-specific epitopes by using CD-specific antisera. *Branhamella catarrhalis* strain 25240, obtained from the American Type Culture Collection (ATCC) was used as the source of
- 25 bacterial genomic DNA. *B. catarrhalis* was grown on chocolate agar plates at 37°C in 5% CO₂ or in brain heart infusion broth. *Escherichia coli* (*E. coli*) Y1090 was used as the host strain for the bacteriophage lambda gt11 genomic library. Depending on the circumstances,
- 30 *E. coli* was grown in LB broth, or on LB agar containing 50 µg/ml of ampicillin. Monoclonal antibodies used for immunoscreening clones included 5E8 and 7D6 which recognize different epitopes on the CD outer membrane protein of *B. catarrhalis* (Sarwar et al., *supra*).
- 35 Antibody 5E8 is an IgM and recognizes an epitope which is exposed on the surface of the intact bacterium.

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Antibody 7D6 is an IgG2a and binds an epitope which is not accessible on the bacterial surface.

5 A lambda gt11 library was constructed with genomic DNA of *B. catarrhalis* 25240 using previously described methods (Nelson et al., 1988, *Infect. Immun.* 56:128-134). Genomic DNA fragments of 2 to 8 kilobases (kb) in size were eluted from an agarose gel and ligated to phage arms. A portion of the library was introduced into *E. coli* Y1090 and the resultant plaques were transferred onto nitrocellulose discs and immunoscreened with monoclonal antibodies 5E8 and 7D6. After incubation with the monoclonal antibodies overnight, the discs were incubated with protein A peroxidase and anti-mouse IgM peroxidase conjugate, with subsequent substrate development, to identify immunoreactive plaques. Screening of a total of approximately 554,000 plaques yielded a clone which contained a 387 base pair insert expressing the epitopes recognized by antibodies 7D6 and 5E8. Nucleotide sequence analysis of the insert contained within this clone showed an open reading frame with no start or stop codons (SEQ ID No. 1). The nucleotide sequence of this clone corresponds to nucleotides 775-1160 of SEQ ID NO. 14 that contains the whole gene sequence encoding CD. The peptide produced by this clone, as shown in SEQ ID NO. 1, corresponds to amino acids 203-331 in the mature protein depicted in SEQ ID NO. 14.

Since several rounds of screening of the lambda gt11 genomic library yielded a small fragment of the CD gene, an EMBL3 library was constructed with genomic DNA of *B. catarrhalis* 25240 with insert sizes of approximately 9 to 23 kb. This library was immunoscreened with monoclonal antibodies 5E8 and 7D6. The EMBL3 genomic library was constructed with methods known in the art (Ausubel et al., 1989, *Current Protocols in Molecular Biology*, published by John Wiley

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and Sons) and according to the recommendations of the manufacturer (Stratagene, LaJolla, CA). Briefly, genomic DNA of *B. catarrhalis* 25240 was purified using SDS, proteinase K and CTAB. The purified genomic DNA was partially digested with Sau3A to generate varying-size fragments. The DNA fragments were separated by sucrose gradient centrifugation on a 10 to 40% sucrose gradient. The fractions containing fragments of approximately 9 to 23 kilobases in size were dephosphorylated using calf alkaline phosphatase, and precipitated with ethanol to prepare for ligation to EMBL3 arms. Approximately 0.7 µg of these genomic DNA fragments were ligated to 1 µg of EMBL3 arms by using T4 DNA ligase. The ligated phage arms and inserts were packaged into phage and the titer of the library was determined by plating on *E. coli* P2 392, the host strain for the lambda EMBL3 genomic library. The EMBL3 genomic library was immunoscreened with monoclonal antibodies 5E8 and 7D6 as described above.

Immunoscreening of approximately 3500 plaques from the EMBL library yielded a single reactive plaque, designated clone 5. The purified clone was assayed with antibodies 5E8 and 7D6 individually and was reactive with both antibodies. Control experiments showed that the protein A and anti-mouse IgM peroxidase conjugates did not bind to plaques of clone 5.

Phage DNA from clone 5 was purified and digested with Sall to excise the insert. Agarose gel electrophoresis revealed that clone 5 had an insert of 13 kb. The insert was digested with several restriction enzymes and a Southern blot assay was performed. The blot was probed with an oligonucleotide corresponding to DNA sequence from the 387 bp fragment of the CD gene recovered from the lambda gt11 library. The gene encoding CD was determined to be localized to a 2.4 kb NcoI-Sall fragment. The 2.4 kb fragment was subcloned

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into the BamHI site of pET11b (Novagen, Madison, WI) by ligating BamHI linkers onto the insert after its ends were made blunt with Klenow DNA polymerase. The resulting plasmid, which contained a 2.4 kb insert, was called pCD1 (Fig. 1). Plasmid pET11b, and recombinant pCD1 were propagated in *E. coli* HB101 on LB agar containing 50 µg/ml of ampicillin. A whole cell lysate of transformants containing pCD1 was subjected to SDS-PAGE and immunoblot assay with antibodies 7D6 and 5E8. The results indicate that pCD1 encodes a full length CD protein which is reactive with both antibodies.

Dideoxy sequencing of both strands of 1727 bp of the 2.4 kb insert of pCD1 was performed with the aid of additional oligonucleotides synthesized to correspond to sequence at appropriate intervals within the insert such as represented by SEQ ID NOS. 2-13. An open reading frame of 453 amino acids, which represents a protein of 48,277 daltons, was identified (SEQ ID NO. 14). A strong transcriptional terminator was present beginning 54 bp downstream of the stop codon.

The calculated molecular mass of the mature protein (45,788 daltons) differed significantly from the apparent molecular mass of OMP CD observed in SDS-PAGE (60,000 or 55,000, daltons in reduced or nonreduced form, respectively). Therefore, a plasmid containing the open reading frame without downstream sequence was constructed to determine whether expression of the reading frame would yield a full size CD protein. A Clal site is located 48 bp downstream of the open reading frame. A BamHI-Clal DNA fragment of 1558 bp containing the putative CD gene was subcloned into pGEM7zf- (Promega Corp., Madison, WI) in constructing new plasmid pCD2 (Fig. 2). By immunoblot assay, shown in Fig. 3 (lane b), *E. coli* transformants containing pCD2 express a full-size CD protein. In addition, the

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immunoblot assay shows that the CD gene product migrates as a doublet (lane b), indicating that both bands represent products of a single gene rather than representing two related proteins produced by their
5 respective genes.

Thus, this embodiment illustrates that nucleotide sequences encoding CD or portions thereof, can be inserted into, and expressed by various vectors including phage vectors and plasmids. Successful
10 expression of the protein and peptides requires that either the insert comprising the gene or gene fragment, or the vector itself, contain the necessary elements for transcription and translation which is compatible with, and recognized by the particular host system used for
15 expression. DNA encoding CD protein, CD peptides, or CD oligopeptides can be synthesized or isolated and sequenced using the methods and primer sequences as illustrated according to Embodiments A, B, and D herein. A variety of host systems may be utilized to express CD
20 protein, CD peptides or CD oligopeptides, which include, but are not limited to bacteria transformed with a bacteriophage vector, plasmid vector, or cosmid DNA; yeast containing yeast vectors; fungi containing fungal vectors; insect cell lines infected with virus (e.g.
25 baculovirus); and mammalian cell lines transfected with plasmid or viral expression vectors, or infected with recombinant virus (e.g. vaccinia virus, adenovirus, adeno-associated virus, retrovirus, etc.).

Using methods known in the art of molecular
30 biology, including methods described above, various promoters and enhancers can be incorporated into the vector or the DNA sequence encoding CD amino acid sequences, i.e. recombinant outer membrane protein CD, CD peptide or CD oligopeptide, to increase the
35 expression of the CD amino acid sequences, provided that the increased expression of the CD amino acid sequences

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is compatible with (for example, non-toxic to) the particular host cell system used. Thus and importantly, the DNA sequence can consist of the gene encoding CD protein, or any segment of the gene which encodes a functional epitope of the CD protein. Further, the DNA can be fused to DNA encoding other antigens, such as other bacterial outer membrane proteins, or other bacterial, fungal, parasitic, or viral antigens to create a genetically fused (sharing a common peptide backbone) multivalent antigen for use as an improved vaccine composition.

The selection of the promoter will depend on the expression system used. Promoters vary in strength, i.e. ability to facilitate transcription. Generally, for the purpose of expressing a cloned gene, it is desirable to use a strong promoter in order to obtain a high level of transcription of the gene and expression into gene product. For example, bacterial, phage, or plasmid promoters known in the art from which a high level of transcription has been observed in a host cell system comprising *E. coli* include the lac promoter, trp promoter, recA promoter, ribosomal RNA promoter, the P_R and P_L promoters, lacUV5, ompF, bla, lpp, and the like, may be used to provide transcription of the inserted DNA sequence encoding CD amino acid sequences.

Additionally, if CD protein, CD peptides, or CD oligopeptides may be lethal or detrimental to the host cells, the host cell strain/line and expression vectors may be chosen such that the action of the promoter is inhibited until specifically induced. For example, in certain operons the addition of specific inducers is necessary for efficient transcription of the inserted DNA (e.g., the lac operon is induced by the addition of lactose or isopropylthio-beta-D-galactoside). A variety of operons such as the trp operon, are under different control mechanisms. The trp operon is induced when

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tryptophan is absent in the growth media. The P_L promoter can be induced by an increase in temperature of host cells containing a temperature sensitive lambda repressor. In this way, greater than 95% of the promoter-directed transcription may be inhibited in uninduced cells. Thus expression of recombinant CD protein, CD peptides, or CD oligopeptides may be controlled by culturing transformed or transfected cells under conditions such that the promoter controlling the expression from the inserted DNA encoding CD amino acid sequences is not induced, and when the cells reach a suitable density in the growth medium, the promoter can be induced for expression from the inserted DNA.

Other control elements for efficient gene transcription or message translation include enhancers, and regulatory signals. Enhancer sequences are DNA elements that appear to increase transcriptional efficiency in a manner relatively independent of their position and orientation with respect to a nearby gene. Thus, depending on the host cell expression vector system used, an enhancer may be placed either upstream or downstream from the inserted DNA sequences encoding CD amino acid sequences to increase transcriptional efficiency. As illustrated previously in this embodiment, other specific regulatory sequences have been identified which may effect the expression from the gene encoding CD. These or other regulatory sites, such as transcription or translation initiation signals, can be used to regulate the expression of the gene encoding CD, or gene fragments thereof. Such regulatory elements may be inserted into DNA sequences encoding CD amino acid sequences or nearby vector DNA sequences using recombinant DNA methods described herein for insertion of DNA sequences.

Accordingly, *B. catarrhalis* nucleotide sequences containing regions encoding for CD protein, CD peptides,

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or CD oligopeptides can be ligated into an expression vector at a specific site in relation to the vector's promoter, control, and regulatory elements so that when the recombinant vector is introduced into the host cell, the *B. catarrhalis* CD-specific DNA sequences can be expressed in the host cell. For example, the CD-specific DNA sequences containing its own regulatory elements can be ligated into an expression vector in a relation or orientation to the vector promoter, and control elements which will allow for expression of CD amino acid sequences. The recombinant vector is then introduced into the appropriate host cells, and the host cells are selected, and screened for those cells containing the recombinant vector. Selection and screening may be accomplished by methods known in the art including detecting the expression of a marker gene (e.g., drug resistance marker) present in the plasmid, immunoscreening for production of CD-specific epitopes using antisera generated to CD-specific epitopes, and probing the DNA of the host's cells for CD-specific nucleotide sequences using one or more oligonucleotides and methods described according to Embodiment C herein.

Genetic engineering techniques may also be used to characterize, modify and/or adapt the encoded CD peptides or CD protein. For example, site-directed mutagenesis to modify an outer membrane protein fragment in regions outside the protective domains, may be desirable to increase the solubility of the subfragment to allow for easier purification. Further, genetic engineering techniques can be used to generate DNA sequences encoding a portion of the amino acid sequence of CD. For example, from the sequence disclosed as SEQ ID NO.14, it can be determined which restriction enzyme or combination of restriction enzymes may be used to generate sequences encoding CD peptides or CD oligopeptides. Restriction enzyme selection may be done

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so as not to destroy the immunopotency of the resultant peptide or oligopeptide. Antigenic sites of a protein may vary in size but can consist of from about 7 to about 14 amino acids. Thus, a protein the size of CD may contain many discrete antigenic sites; therefore, many partial gene sequences could encode antigenic epitopes of CD. Consequently, using for example SEQ ID NO.14 as a guide, restriction enzyme combinations may be used to generate DNA sequences, which when inserted into the appropriate vector, are capable of directing the production of CD-specific amino acid sequences (protein, peptides or oligopeptides) comprising one or more antigenic epitopes.

15 Embodiment B

Conservation of the gene encoding CD amongst *B. catarrhalis* strains.

For the nucleotide sequences of the present invention to be useful in diagnostic assays, the gene encoding CD must be highly conserved amongst strains of *B. catarrhalis*. In addition, a highly conserved gene indicates that the protein sequence is also highly conserved. For a bacterial protein or peptide to be useful as an antigen in subunit vaccine formulations against infection caused by *B. catarrhalis*, the protein or peptide must contain epitopes that are both immunogenic, and conserved amongst strains of *B. catarrhalis*. To determine the degree of conservation of the CD gene among strains of *B. catarrhalis*, genomic DNA was purified and analyzed from 30 isolates recovered from diverse clinical and geographic sources (Table 1). Analysis involved restricting the DNA into fragments, and probing with an oligonucleotide of a sequence which includes , but is not limited to, those represented by SEQ ID NOs. 2-13.

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Table 1.

Sources of isolates of *Branhamella catarrhalis*

<u>Clinical Site</u>	<u>Number of Isolates</u>
sputum	15
5 middle ear fluid ¹	7
nasopharynx	3
eye	2
adenoid	1
blood	1
10 <u>ATCC²</u>	<u>1</u>
1 Middle ear fluid was obtained by tympanocentesis.	
2 American Type Culture Collection.	

The genomic DNA from 30 strains (including 25240)
 of *B. catarrhalis* was purified. A volume of 30 ml of
 brain heart infusion broth was inoculated with a single
 colony and incubated at 37°C with shaking overnight.
 Cells were harvested by centrifugation at 2200 x g for
 10 minutes at 4°C. The pelleted cells were suspended in
 7 ml of TE buffer (0.01 M Tris, pH 8, 0.001 M EDTA, pH
 8.0). EDTA was added to 0.005 M and SDS was added to
 0.5%. The suspension was incubated at 60° for 30
 minutes. Proteinase K was added to 200 µg/ml followed
 by incubation at 37°C for approximately 24 hours. The
 sample was extracted sequentially with equal volumes of
 phenol, followed by phenol/ chloroform at a 1:1 ratio,
 followed by chloroform. A 10% volume of 3 M sodium
 acetate (pH 5.2) was added and DNA was precipitated by
 the addition of cold ethanol equivalent to 80% of the
 volume. Genomic DNA precipitated and was removed by
 "spooling" with a pasteur pipette. The DNA was washed
 in 70% ethanol and dissolved in 0.05 M tris, pH 8.0.
 RNase was added to a final concentration of 40 µg/ml and
 the sample was incubated at 37° for 30 minutes. EDTA
 was added to a concentration of 0.001 M. The sample was
 extracted sequentially with phenol and chloroform and

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ethanol precipitated. The purified DNA was dissolved in 0.01 M Tris, 0.1 mM EDTA, pH 8.0.

An aliquot equivalent to 10 μ g of DNA was digested with EcoRI or PstI with a reaction volume of 0.5 ml.

- 5 The resulting DNA fragments were separated by agarose gel electrophoresis and transferred to a charged nitrocellulose membrane by Southern blot. The Southern blots were probed with two oligonucleotide probes corresponding to sequences upstream and downstream of the EcoRI site within the gene encoding CD (this Eco RI site is depicted in Fig. 1). The oligonucleotides had been end-labeled with [32 P]ATP by using T4 polynucleotide kinase before use as probes. Hybridizations were carried out at 37°C and washes were performed at 48°C.
- 10 The hybridization and wash buffers were described previously (Nelson et al., supra). Autoradiography was performed at -70°C.

- All 30 strains produced an identical pattern of bands, including a 325 bp band representing the fragment between the EcoRI site within the gene and the site just downstream of the gene. In addition, all 30 strains showed a 3.7 kb band representing a fragment upstream of the CD gene. This pattern is exemplified by Fig. 4 showing the Southern blot assay of 13 strains.
- 20

- 25 To further analyze the molecular conservation of the CD gene, genomic DNA from the same 30 strains was digested with EcoRI and PstI and probed with oligonucleotides. Figure 1 shows that the gene encoding CD, isolated from strain 25240, has a PstI site near the center of the gene and that digestion with EcoRI and PstI will yield a DNA fragment of 337 base pairs.
- 30 Southern blot assays showed that genomic DNA from 30 of 30 strains of *B. catarrhalis* yielded an identical 337 bp fragment which hybridized with an oligonucleotide corresponding to sequence in the gene encoding CD isolated from strain 25240. This pattern is exemplified
- 35

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by Fig. 5 showing the Southern blot assay of 13 strains. These findings indicate that the gene encoding CD is highly conserved amongst strains of *B. catarrhalis*, and therefore the nucleotide sequences described herein have applications for diagnostic and vaccine use.

Embodiment C

Methods for using CD-specific nucleotide sequences in molecular diagnostic assays for the detection of *B. catarrhalis*.

Because of the conservation of the gene encoding CD, as disclosed in Embodiment B, the nucleic acid sequences of the present invention can be used in molecular diagnostic assays for detecting *B. catarrhalis* genetic material. In particular, CD sequence-specific oligonucleotides can be synthesized for use as primers and/or probes in amplifying, and detecting amplified, nucleic acids from *B. catarrhalis*. Recent advances in molecular biology have provided several means for enzymatically amplifying nucleic acid sequences. Currently the most commonly used method, PCR™ (polymerase chain reaction, Cetus Corporation) involves the use of Taq Polymerase, known sequences as primers, and heating cycles which separate the replicating deoxyribonucleic acid (DNA) strands and exponentially amplify a gene of interest. Other amplification methods currently under development include LCR™ (ligase chain reaction, BioTechnica International) which utilizes DNA ligase, and a probe consisting of two halves of a DNA segment that is complementary to the sequence of the DNA to be amplified; enzyme QB replicase (Gene-Trak Systems) and a ribonucleic acid (RNA) sequence template attached to a probe complementary to the DNA to be copied which is used to make a DNA template for exponential production of complementary RNA; and NASBA™ (nucleic acid sequence-based amplification, Cingene Corporation)

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which can be performed on RNA or DNA as the nucleic acid sequence to be amplified.

Nucleic acid probes that are capable of hybridization with specific gene sequences have been used successfully to detect specific pathogens in biological specimens at levels of sensitivity approaching 10^3 - 10^4 organisms per specimen (1990, Gene Probes for Bacteria, eds. Macario and deMacario, Academic Press). Coupled with a method that allows for amplification of specific target DNA sequences, species-specific nucleic acid probes can greatly increase the level of sensitivity in detecting organisms in a clinical specimen. Use of these probes may allow direct detection without relying on prior culture and/or conventional biochemical identification techniques. This embodiment of the present invention is directed to primers which amplify species-specific sequences of the gene encoding CD of *B. catarrhalis*, and to probes which specifically hybridize with these amplified DNA fragments. By using the nucleic acid sequences of the present invention and according to the methods of the present invention, as few as one *B. catarrhalis* organism may be detected in the presence of 10 μ g/ml extraneous DNA.

This embodiment is directed to species-specific oligonucleotides which can be used to amplify sequences of *B. catarrhalis* DNA, if present, from DNA extracted from clinical specimens including middle ear fluid, sputum, blood, and fluids from the nasopharynx, eye, and adenoid; and to subsequently determine if amplification has occurred. In one embodiment of the present invention, a pair of *B. catarrhalis*-specific DNA oligonucleotide primers are used to hybridize to *B. catarrhalis* genomic DNA that may be present in DNA extracted from a clinical specimen, and to amplify the specific segment of genomic DNA between the two flanking

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primers using enzymatic synthesis and temperature cycling. Each pair of primers are designed to hybridize only to the *B. catarrhalis* nucleotide sequences of the present invention to which they have been synthesized to complement; one to each strand of the double-stranded DNA. Thus, the reaction is specific even in the presence of microgram quantities of heterologous DNA. For the purposes of this description, the primer derived from the sequence of the positive (gene) strand of DNA will be referred to as the "positive primer", and the primer derived from the sequence of the negative (complementary) strand will be referred to as the "negative primer".

Amplification of DNA may be accomplished by any one of the methods commercially available. For example, the polymerase chain reaction may be used to amplify the DNA. Once the primers have hybridized to opposite strands of the target DNA, the temperature is raised to permit replication of the specific segment of DNA across the region between the two primers by a thermostable DNA polymerase. Then the reaction is thermocycled so that at each cycle the amount of DNA representing the sequences between the two primers is doubled, and specific amplification of the *B. catarrhalis* DNA sequences, if present, results. Further identification of the amplified DNA fragment, as being derived from *B. catarrhalis* DNA, may be accomplished by liquid hybridization. This test utilizes one or more labeled oligonucleotides as probes to specifically hybridize to the amplified segment of *B. catarrhalis* DNA. Detection of the presence of sequence-specific amplified DNA may be accomplished using any one of several methods known in the art such as a gel retardation assay with autoradiography. Thus, the nucleotide sequences of the present invention provide basis for the synthesis of oligonucleotides which have commercial applications in

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diagnostic kits for the detection of *B. catarrhalis*. In a related embodiment, the oligonucleotides used as primers may be labeled directly, or synthesized to incorporate label. Depending on the label used, the amplification products can then be detected, after binding onto an affinity matrix, using isotopic or colorimetric detection.

DNA may be extracted from a clinical specimens which may contain *B. catarrhalis* using methods known in the art. For example, cells contained in the specimen may be washed in TE buffer and pelleted by centrifugation. The cells then may be resuspended in 100 μ l of amplification reaction buffer containing detergents and proteinase K. Using the polymerase chain reaction, the resultant sample may be composed of the cells in 10mM Tris pH 8.3, 50mM KCl, 1.5mM MgCl₂, 0.01% gelatin, 0.45% NP40™, 0.045% Tween 20™, and 60 μ g/ml proteinase K. The sample is incubated at 55°C water bath for 1 hour. Following the incubation, the sample is incubated at 95°C for 10 minutes to heat-inactivate the proteinase K. The sample may then be amplified in accordance with the protocol for the polymerase chain reaction as set forth below.

The *B. catarrhalis* DNA may be amplified using any one of several protocols for amplifying nucleic acids by the polymerase chain reaction. In one mode of this embodiment, the gene encoding CD was amplified from 25 clinical isolates of *B. catarrhalis* using the following conditions. DNA to be amplified (\sim 1 μ g of genomic DNA) was distributed in 0.5 ml microfuge tubes and the volume was adjusted to 50 μ l by adding a reaction mixture comprising 0.2 mM dNTPs (dATP, dCTP, dGTP, dTTP), 0.25 μ g of each positive and negative oligonucleotide primer, 1 unit of TaqI polymerase, TaqI 10x buffer (5 μ l), 1mM MgCl₂ (final concentration), and sterile distilled water to achieve the total volume. The TaqI polymerase is added

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to the reaction mixture just before use and is gently mixed, not vortexed. A layer of mineral oil, approximately 2 drops, is added to each tube and then the tubes are placed in the thermal cycler. Thirty to
5 thirty-five cycles are generally sufficient for bacterial DNA amplification. One cycle consists of 1 minute at 95°C, 1 minute at 37°C, and 1 minute at 72°C. The first cycle includes a 1½ minute incubation at 95°C to assure complete denaturation.

10 Oligonucleotides useful as primers or probes which specifically hybridize to the gene encoding CD of *B. catarrhalis* and used in DNA amplification and/or detection can be biochemically synthesized, using methods known in the art, from the nucleotide sequences
15 disclosed in the present invention. The specificity of the oligonucleotides for *B. catarrhalis* can be checked by a genebank database (Genbank) search for each individual sequence. In general, the oligonucleotides should be selected for low G-C content. Pairs of primers
20 that have been used for this embodiment to amplify the whole gene encoding CD include SEQ ID NO. 15 (negative primer) and SEQ ID NO. 16 (positive primer). Pairs of primers used to amplify the portion of the gene that encodes 5E8 and 7D6 epitopes include SEQ ID NO. 17
25 (negative primer) and SEQ ID NO. 18 (positive primer).

For detection purposes, the oligonucleotides of the present invention may be end-labeled with a radioisotope. Probe sequences, internal to the two primers used for amplification of the gene sequence, may
30 be end-labeled using T₄ polynucleotide kinase and gamma ³²P ATP. Twenty pMols of probe DNA in kinase buffer (50mM Tris, pH 7.6, 10mM MgCl₂, 5mM dithiothreitol, 0.1mM spermidine-HCl, 0.1mM EDTA, pH 8.0) is mixed with 120μCi of gamma ³²P ATP and incubated at 37°C for 1 hour. Labeled
35 probe is separated from unincorporated label on an 8% acrylamide gel run for 1 hour at 200 volts in Tris

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Borate EDTA (TBE) buffer at room temperature. Labeled probe is first located by exposing the acrylamide gel to x-ray film for three minutes. The resulting autoradiogram is then positioned under the gel, and the band containing the labeled probe was excised from the gel. The gel slice is pulverized in one milliliter of sterile distilled water, and the probe is eluted by shaker incubation overnight at 37°C. The eluted probe is separated from the gel fragments by centrifugation using a chromatography prep column. Radioactivity of the probe is determined, by counting one microliter of the labeled probe on a glass fiber filter, by liquid scintillation. Such probe sequences may be chosen from any of the sequences identified as SEQ ID NOs. 2-13 provided the probe sequence is internal to the two primers used for amplification of the desired nucleotide sequence disclosed in the present invention.

Alternative methods known in the art may be used to improve the detection of amplified target sequences in accordance with the compositions and methods of the present invention. The sensitivity of detection of the amplified DNA sequences can be improved by subjecting the sequences to liquid hybridization. Alternative methods of detection known in the art, in addition to gel electrophoresis and gel electrophoresis with Southern hybridization and autoradiography, that may be used with the compositions and methods of the present invention include: restriction enzyme digestion with gel electrophoresis; slot-blot hybridization with a labeled oligonucleotide probe; amplification with a radiolabeled primer with gel electrophoresis, Southern hybridization and autoradiography; amplification with a radiolabeled primer with dot blot and autoradiography; amplification with oligonucleotides containing affinity tags (ex. biotin, or one primer incorporating biotin and the other primer with a sequence specific for a DNA binding

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protein) followed by detection in an affinity-based assay (ex. ELISA); and amplification with oligonucleotides containing fluorophores followed by fluorescence detection.

5 One embodiment of non-isotopic detection involves incorporating biotin into the oligonucleotide primers of the present invention. The 5'-aminogroup of the primers may be biotinylated with sulfo-NHS-biotin, or biotin may be incorporated directly into the primer by synthesizing
10 the primer in the presence of biotin-labeled dNTPs. The non-isotopic labeled primers are then used in amplifying DNA from a clinical specimen. The detection for the presence or absence of amplified target sequences may be accomplished by capturing the amplified target sequences
15 using an affinity matrix having avidin bound thereto, followed by incubation with an avidin conjugate containing an enzyme which can be used to visualize the complex with subsequent substrate development. Alternately, the amplified target sequences may be
20 immobilized by hybridization to the corresponding probes of the target sequence wherein the probes have been affixed onto a matrix. Detection may be accomplished using an avidin conjugate containing an enzyme which can be used to visualize the complex with subsequent
25 substrate development.

Embodiment D

Characterization of CD, including generation of CD peptides.

30 To confirm that the gene encoding CD had been identified, the amino terminus of the CD protein was determined. To identify the amino terminus of the CD protein, purified outer membrane of *B. catarrhalis* 25240 was subjected to SDS-PAGE and transferred to
35 polyvinylidene difluoride membrane by electrophoretic blotting. The CD band was excised and the amino

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terminal sequence of the protein was determined by Edmund degradation, with the amino acids being analyzed by a microsequencer. The amino terminal sequence, G-V-T-V-S-P-L-L-L-G corresponded to amino acids 27 through 36 of the open reading frame of pCD1, indicating that CD has a 26 amino acid leader peptide. A hydrophobic 26 amino acid leader peptide is characteristic of bacterial OMPs whose leader peptides are cleaved by signal peptidase I (Oliver, 1985, *Ann. Rev. Microbiol.* 39:615-648).

To further establish that the gene encoding CD had been identified, the amino acid sequence deduced from the gene sequence was analyzed for the presence of methionine residues to predict the result of cyanogen bromide cleavage of the protein. The open reading frame corresponding to the mature protein contains four methionines indicating that cleavage with cyanogen bromide would yield five fragments. Cyanogen bromide cleavage of CD was accomplished by purifying outer membrane (Murphy et al., 1989, *Infect. Immun.* 57:2938-2941) and subjecting the outer membrane preparation to SDS-PAGE. The gel was stained with amido black so that the CD band could be visualized and excised from the gel. The gel slices (3-4 mm in length) were placed into the tubes of an electroeluter with 0.05 M ammonium bicarbonate, 0.1% SDS. The protein was eluted, at 10 mA per tube until the gel slices were completely free of amido black (approximately 5 hours). The eluted protein was collected and an aliquot of 0.6 ml was precipitated by the addition of 2 ml of cold ethanol. The sample was centrifuged and the pellet was air dried. A volume of 0.4 ml of cyanogen bromide (200 mg/ml) in 70% formic acid was added to the pellet and the sample was incubated overnight at room temperature in the dark. A simultaneous control sample was incubated in 70% formic acid under identical conditions. The next day 1 ml of

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water was added and the samples were lyophilized. The lyophilized peptides were suspended in sample buffer and subjected to tricine gel electrophoresis.

Table 2 shows the size of the fragments (CD peptides) predicted by the methionine sites in the open reading frame. Figure 6 shows the actual fragments obtained from cyanogen bromide treatment of purified CD, as determined by the tricine polyacrylamide gel system of Lesse et al. (1990, *J. Immunol. Methods* 126:109-117). The predicted and actual sizes of the cyanogen bromide cleaved fragments are in good agreement with the exception of the large fragment from the amino terminal region of the protein (Table 2).

Table 2.

Cyanogen bromide cleavage fragments of outer membrane protein CD of *Branhamella catarrhalis*

	Molecular mass ¹ predicted from gene sequence ²	Molecular mass measured from SDS-PAGE ³
20	34,919	~50,000
	4,408	4,900
	3,593	4,000
	2,450	2,500
	358	

-
- 1 Molecular masses are noted in daltons.
 - 2 See SEQ ID No. 14 for nucleotide sequence.
 - 3 SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis. See Figure 6 for tricine gels.
-

Thus, the open reading frame identified in pCD1 represents the entire gene encoding CD and the protein behaves aberrantly in SDS-PAGE. This discrepancy between the predicted molecular mass and the molecular mass observed in SDS-PAGE appears to be due to a proline-rich region in the large cyanogen bromide

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fragment in the amino terminal region of the protein as a variety of other proline-rich proteins demonstrate this characteristic (Postle et al., 1983, *Proc. Natl. Acad. Sci. USA* 50:5235-5239; Woods et al., 1989, *Mol. Microbiol.* 3(1):43-48; and Thole et al., 1990, *Infect. Immun.* 58:80-87).

A search of sequence data bases disclosed that the sequence of the CD gene shares homology with the OprF genes of *Pseudomonas* species. The CD protein contains a region (amino acid 240-280) which is rich in proline, alanine and valine. This sequence shares homology with the TonB protein of *E. coli* and *Serratia marcescens*.

Embodiment E

Methods for using CD, or CD peptides, in diagnostic immunoassays.

CD protein, CD peptides, and CD oligopeptides can be purified for use as immunogens in vaccine formulations; and as antigens for diagnostic assays or for generating *B. catarrhalis* specific antisera of therapeutic and/or diagnostic value. CD protein from *B. catarrhalis* or oligopeptides or peptides thereof, or recombinant CD protein, recombinant CD peptides, or recombinant CD oligopeptides produced from an expression vector system, can be purified with methods known in the art including detergent extraction, chromatography (e.g., ion exchange, affinity, immunoaffinity, or sizing columns), differential centrifugation, differential solubility, or other standard techniques for the purification of proteins.

1) For example, a partially purified preparation, containing primarily bacterial outer membrane proteins, can be prepared as follows. *B. catarrhalis* cultures from 30 chocolate agar plates were scraped into 25 ml of PBS, pH 7.2, and harvested by centrifugation at 12,000 x g for 20 minutes at 4°C. The bacterial pellet was

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resuspended in 10 ml of 1 M sodium acetate-0.001 M β -mercaptoethanol (pH 4.0). A 90-ml volume of a solution containing 5% Zwittergent Z 3-14 (Calbiochem-Behring) and 0.5% M CaCl₂ was added, and the suspension was mixed
5 for 1 hour at room temperature. Nucleic acids were precipitated by the addition of 25 ml cold ethanol and subsequent centrifugation at 17,000 x g for 10 minutes at 4°C. The remaining proteins were precipitated by the addition of 375 ml cold ethanol and collected by
10 centrifugation at 17,000 x g for 20 minutes at 4°C. The pellets were allowed to dry and were then suspended in 10 ml of detergent buffer containing 0.05% Zwittergent, 0.05 M Tris, 0.01 M EDTA, pH 8.0, and mixed for 1 hour at room temperature. The bacterial outer membrane
15 proteins are present in the soluble fraction of the detergent buffer after centrifugation at 12,000 x g for 10 minutes at 4°C.

Immunopurification of the CD protein from an outer membrane protein preparation may be accomplished using
20 methods known in the art for immunoaffinity chromatography. CD-specific monoclonal antibodies, such as 5E8 and 7D6, may be linked to a chromatographic matrix to form an affinity matrix. The outer membrane protein preparation is then incubated with the affinity
25 matrix allowing the antibodies to bind to CD. The affinity matrix is then washed to remove unbound components and CD is then eluted from the affinity matrix resulting in a purified preparation of CD protein. The purified CD may be used as an antigen for
30 diagnostic assays, or may be chemically or enzymatically cleaved into peptides, as illustrated in Embodiment D. Alternatively, CD peptides may be synthesized using the deduced amino acid sequence from the gene encoding CD as a reference.

35 2) In another illustration of this embodiment, recombinant CD was purified from a polyhistidine

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expression plasmid. To purify recombinant CD by this method, the gene encoding CD was cloned into a polyhistidine expression vector such as plasmid pRSETA (Invitrogen Corporation), such that upon expression
5 several histidine residues ("polyhistidine tail") are attached to the amino terminus of the CD protein. A BamHI fragment containing the gene encoding CD was ligated into the expression vector which had been previously restricted with BamHI and subsequently
10 treated with calf intestinal phosphatase. The ligation mixture was used to electroporate *E. coli* strain BL21(DE3) cells, and transformants were analyzed for recombinant plasmids containing the gene encoding CD in the proper orientation with respect to the plasmid
15 promoter. One such clone, termed pCDSA, was isolated and was also shown to express CD protein when introduced into the *E. coli* host strain.

Recombinant CD was purified as follows. A 15 ml volume of a culture of transformants containing pCDSA
20 was grown overnight in LB ampicillin broth at 37°C. The following morning, 135 ml of broth was inoculated with the overnight culture and grown for 1 hour at 37°C. Cells were recovered by centrifugation at 5,000 x g for 10 minutes at 4°C. Cells were resuspended in 10 ml of
25 guanidinium lysis buffer (6 M guanidine hydroxide, 20 mM sodium phosphate, 500 mM sodium chloride, pH 7.8). The suspension was mixed for 10 minutes at room temperature. Cells were then sonicated with 3 bursts of 5 seconds each using a sonifier at the #4 setting. The mixture was
30 centrifuged at 3,000 x g for 15 minutes at 4°C and the supernatant was saved. The supernatant was then mixed for 10 minutes at room temperature with 1.6 ml of a resin (e.g., ProBond™, Invitrogen) which, via nickel on the resin, binds to the polyhistidine tail of the
35 recombinant CD protein. The resin was then isolated by centrifugation.

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CD protein was eluted from the resin by first washing the resin twice with 4 ml of denaturing wash buffer (8 M urea, 20 mM sodium phosphate, 500 mM sodium chloride, pH 7.8). The resin was then washed 2 times with 4 ml volumes of denaturing wash buffer at pH 6.0. This was followed by washing the column twice with 4 ml volumes of denaturing wash buffer at pH 4.0. Fractions of 1 ml each were collected and dialyzed against phosphate-buffered saline (PBS) containing a detergent (0.1% Triton X-100). Analysis of the eluted CD protein by gel electrophoresis and Coomassie blue staining revealed a single band. It is estimated that this method results in a preparation of CD protein which is 95% purified. The resultant purified recombinant CD protein is immunoreactive with monoclonal antibodies which recognize native CD protein.

3) In another illustration of this embodiment, native CD protein was purified from *B. catarrhalis*. *B. catarrhalis* isolate O35E was grown in Mueller-Hinton broth for 24 hours at 36°C. The bacteria were then harvested by centrifugation at 4,000 x g for 20 minutes. The bacterial pellets were suspended in pH 7.0 buffer containing 0.01 M phosphate and 0.64 M sodium chloride. The resuspended bacteria were vortexed vigorously for 2 minutes, and then centrifuged at 30,000 x g for 20 minutes. The supernatant, containing outer membrane vesicles, was saved and dialyzed against a solution containing 10 mM Tris, 10 mM NaCl and 1 mM EDTA. After dialysis, a detergent (Triton X-100) was added to a concentration of 1.0%, and the dialysate was then incubated for 1 hour at room temperature to solubilize the proteins. Insoluble material was removed by centrifugation at 10,000 x g for 10 minutes. The supernatant was loaded onto an ion exchange column. The column was washed with a buffer of 10 mM Tris, 1% Triton X-100 (pH 8.0) and the protein eluted with the buffer

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containing either 50 mM NaCl or 100mM NaCl. Subsequent passage of the eluted protein preparation over a gel filtration column, that had been equilibrated with the buffer, yielded preparations of protein that were free of other detectable proteins by Coomassie Brilliant Blue staining of SDS-PAGE gels, and free of lipooligosaccharides by silver staining of SDS-PAGE gels.

4) As used throughout the specification, CD oligopeptides are defined herein as a series of peptides corresponding to a portion of the amino acid sequence of CD protein as disclosed in SEQ ID NO.14 that are synthesized as one or chemically-linked. Such peptides or oligopeptides can be synthesized using one of the several methods of peptide synthesis known in the art including standard solid peptide synthesis using tert-butyloxycarbonyl amino acids (Mitchell et al., 1978, *J. Org. Chem.* 43:2845-2852), using 9-fluorenylmethyloxycarbonyl amino acids on a polyamide support (Dryland et al., 1986, *J. Chem. Soc. Perkin Trans. I*, 125-137); by pepscan synthesis (Geysen et al., 1987, *J. Immunol. Methods* 03:259; 1984, *Proc. Natl. Acad. Sci. USA* 81:3998); by standard liquid phase peptide synthesis; or by recombinant expression vector systems. Modification of the peptides or oligopeptides, such as by deletion and substitution of amino acids (and including extensions and additions to amino acids) and in other ways, may be made so as to not substantially detract from the immunological properties of the peptide or oligopeptide. In particular, the amino acid sequence of the CD protein, or peptide or oligopeptide thereof, may be altered by replacing one or more amino acids with functionally equivalent amino acids resulting in an alteration which is silent in terms of an observed difference in the physicochemical behavior of the protein, peptide, or oligopeptide. Functionally

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equivalent amino acids are known in the art as amino acids which are related and/or have similar polarity or charge. Thus, an amino acid sequence which is substantially that of the amino acid sequences depicted in the Sequence Listing herein, refers to an amino acid sequence that contains substitutions with functionally equivalent amino acids without changing the primary biological function of protein, peptide, or oligopeptide.

5 5) In an illustration of production of CD peptides containing epitopes of CD, defined regions of the CD protein were expressed in an expression system wherein the plasmid expression vector (pGEX2T) directs the synthesis of foreign polypeptides in *E. coli* as a fusion peptides with glutathione-S-transferase (GST), a 26 kilodalton protein from *Schistosoma japonicum*. In this mode of the embodiment, and using pCD2 as the template, selected regions of the gene encoding CD was amplified by the polymerase chain reaction using oligonucleotides corresponding to sequences shown in Table 3. Table 3 also shows the amino acid positions of the peptides, relative to the mature CD protein as illustrated in SEQ ID NO.14, as encoded by the peptide constructs. The oligonucleotides were designed so that the resulting amplified gene fragment contained a *Bam*H1 restriction site on its 5' end, and an *Eco*R1 restriction site on the 3' end so that the amplified fragment could be directionally cloned into pGEX2T. The sequence of each recombinant plasmid was confirmed by dideoxy sequencing.

10 To purify each recombinant CD peptide, the respective recombinant plasmid containing the gene fragment was transformed into *E. coli* JM109. The transformant was grown in 400 ml of LB broth containing 25µg/ml of ampicillin by adding forty ml of an overnight culture to 360 ml of broth, and incubating for 1.5 hours at 37°C with shaking. IPTG was added to 0.01mM and the culture

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was incubated for an additional 3 hours. Cells were centrifuged at 5000 x g and the cell pellet was resuspended in 5 ml of PBS. Cells were sonicated and the mixture was centrifuged at 10,000 x g for 10 minutes.

- 5 The supernatant was mixed with 0.5 ml of preswelled glutathione-agarose beads. After mixing for 2 minutes at room temperature, the beads (with fusion peptide bound to the glutathione) were washed 2 additional times with PBS containing 1% triton-X-100. The beads were then
- 10 washed once in 0.05M Tris, pH 8.0. To cleave the CD peptide from the glutathione-S-transferase, the washed beads were incubated in 0.25% (final concentration) human thrombin in Tris buffer for 1 hour at room
- 15 temperature. A protease inhibitor, PMSF, was then added to a concentration of 100 µg/ml. The beads were removed by centrifugation and the supernatant contained purified CD peptide. Immunoblot assays affirmed that the fusion peptides were immunoreactive, though at varying degrees with CD-specific rabbit polyclonal antiserum.

20

Table 3

	Clone	Amino Acid positions	Oligonucleotides
	Px1	1-105 (SEQ ID NO. 19)	SEQ ID NOS. 20-21
	Px106	106-202 (SEQ ID NO. 22)	SEQ ID NOS. 23-24
25	Px203	203-261 (SEQ ID NO. 25)	SEQ ID NOS. 26-27
	Px261L	261-331 (SEQ ID NO. 28)	SEQ ID NOS. 29-30
	Px261	261-301 (SEQ ID NO. 31)	SEQ ID NOS. 29 & 32
	Px286L	286-311 (SEQ ID NO. 33)	SEQ ID NOS. 34-35
	Px286	286-301 (SEQ ID NO. 36)	SEQ ID NOS. 34 & 37
30	Px293	293-303 (SEQ ID NO. 38)	SEQ ID NOS. 39-40
	Px295	295-311 (SEQ ID NO. 41)	SEQ ID NOS. 42-43
	Px311	311-331 (SEQ ID NO. 44)	SEQ ID NOS. 45-46
	Px332	332-390 (SEQ ID NO. 47)	SEQ ID NOS. 48-49
	Px391	391-427 (SEQ ID NO. 50)	SEQ ID NOS. 51-52

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Purified CD protein, CD peptides, and CD oligopeptides may be used as antigens in immunoassays for the detection of *Branhamella catarrhalis*-specific antisera present in the body fluid of an individual suspected of having an infection caused by *B. catarrhalis*. The body fluids include, but are not limited to, middle ear fluid, sputum, blood, and fluids from the nasopharynx, eye, and adenoid. The detection of CD or CD peptides as an antigen in immunoassays, includes any immunoassay known in the art including, but not limited to, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), "sandwich" assay, precipitin reaction, agglutination assay, fluorescent immunoassay, and chemiluminescence-based immunoassay.

Embodiment F

Methods and compounds for vaccine formulations related to CD and peptides.

This embodiment of the present invention is to provide CD protein and/or peptides or oligopeptides thereof, to be used in as immunogens in a prophylactic and/or therapeutic vaccine for active immunization to protect against or treat infections caused by *B. catarrhalis*. For vaccine purposes, an antigen of *B. catarrhalis* comprising a bacterial protein should be immunogenic, and induce functional antibodies directed to one or more surface-exposed epitopes on intact bacteria, wherein the epitope(s) are conserved amongst strains of *B. catarrhalis*.

In one illustration of the CD protein having the properties desirable of a vaccine antigen, the protein was purified from *B. catarrhalis* using the method described herein in Embodiment E, Example 3. Mice were immunized with the purified CD protein (25 μ g) with adjuvant (20 μ g of QS21) two times at four week intervals. Blood from the immunized mice was drawn 32

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days after the last immunization and the immune sera was pooled. The pooled immune sera was assayed against whole bacteria (*B. catarrhalis* strain 035E) by an enzyme linked immunosorbent assay (ELISA). For the whole cell ELISA, overnight cultures of bacteria were harvested by a swab and suspended in PBS to an absorbance of 0.1 at 600 nm. Aliquots (100 μ l) of the bacterial suspension were added to the wells of a 96 well microtiter plate and dried overnight at room temperature. The plates were blocked with 100 μ l of 0.1% (w/v) gelatin in PBS. This, and all remaining incubations, were for one hour at room temperature unless otherwise specified. The blocking solution was removed and 100 μ l of the immune sera, diluted in PBS with 0.1% (w/v) gelatin, was added to the wells and incubated. After washing three times with PBS, the bound antibodies were detected by incubating with 100 μ l of alkaline phosphatase conjugated recombinant protein G (1:1500 in PBS with 0.1% (w/v) gelatin). The plates were washed and color development was facilitated by the addition of 100 μ l/well of p-nitrophenyl phosphate (2 mg/ml in diethanolamine). After 30 minutes, the reaction was stopped by adding 50 μ l of 3M NaOH. The absorbance was read at 492 nm using an ELISA reader. Endpoint titers were determined as the reciprocal of the dilution at which the absorbance was greater than that of the blank wells. The results, given in Table 4, demonstrate that immunization with CD protein can elicit antibodies which can bind to one or more surface-exposed epitopes on intact *B. catarrhalis*.

Table 4. Titer of serum against strain 035E

Serum	Endpoint titer
pre-immune control	<50
immune (anti-CD) sera	7,800

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Additional evidence supporting the immunogenicity of the CD protein comes from a study of the human immune response to outer membrane proteins of *B. catarrhalis*, in which 11 of 13 patients with well-documented infections caused by *B. catarrhalis* had IgG to CD in their convalescent sera (unpublished). The 13 patients included 6 children with otitis media and 7 adults with bronchopulmonary infections due to *B. catarrhalis*.

In another illustration that the CD protein possesses properties desirable of a vaccine antigen, it was demonstrated that CD protein is the target of bactericidal antibody generated from immunization with CD protein. For example, polyclonal antiserum to CD protein was raised in a rabbit by immunizing with CD protein subcutaneously. Outer membrane preparations of *B. catarrhalis* were subjected to SDS-PAGE, and bands in the gel corresponding to CD were cut out, and then CD protein was purified by elution from the polyacrylamide gel slices. A rabbit was immunized at day 0 with 40 μ g of CD protein in incomplete Freund's adjuvant, day 14 with 90 μ g of CD protein in incomplete Freund's adjuvant, and day 28 with 60 μ g of CD protein in incomplete Freund's adjuvant. The resultant antiserum was tested for its bactericidal activity against strain 4223NC of *B. catarrhalis*. The bacteria were grown to logarithmic phase in brain-heart infusion (BHI) broth. An aliquot of the bacterial culture was diluted to 5×10^4 colony forming units (CFU) per ml in 10% bovine serum albumin in a balanced salt solution. The bactericidal assay reaction contained bacteria, polyclonal antiserum to CD protein, a complement source consisting of normal human serum which was absorbed with protein G to remove antibodies, and the balanced salt solution. All reagents were added to the reaction to yield a 250 μ l volume. Aliquots of 25 μ l of the reaction were removed

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and plated in triplicate on BHI agar at times 0 and 60 minutes. The plates were incubated and colonies were counted the next day. The percent killing was calculated using the average of the three triplicate values at the 2 times. A representative example of data generated by the bactericidal assays is shown in Table 5. The results indicate that the polyclonal antiserum raised to CD protein is bactericidal for *B. catarrhalis*. As illustrated by Table 5, controls show that the antiserum does not kill bacteria in the absence of complement, and that the complement source does not kill the bacteria in the absence of the antiserum, indicating that the bactericidal activity is antibody directed and complement mediated.

Table 5. Bactericidal activity of anti-CD antibody

Sample	Antiserum	Complement	CFU at time 0	CFU at time 60	percent killing
1	10 μ l	22 μ l	225	5	97.8%
2	10 μ l	0	227	390	0%
3	0	22 μ l	254	286	0%

In further illustrating that CD protein possesses properties desirable of a vaccine antigen, pooled immune sera raised to strain O35E was shown to have cross-reactivity with heterologous strains. The pooled immune sera, prepared against CD protein as described above, was examined for cross-reactivity with nine *B. catarrhalis* strains from diverse clinical and geographical sources. These include strains isolated from clinical sources such as middle ear and from the upper respiratory tract, and from geographical sources such as New York state, Massachusetts, and Tennessee. The assay was performed by culturing the strains overnight on Mueller-Hinton agar. Bacteria from each

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5 culture were harvested by swabs and suspended in PBS to
an optical absorbance of 1.0 at 600 nm. A microliter of
each suspension was applied to a nitrocellulose membrane
and allowed to dry. The membrane was incubated one hour
10 at room temperature in a solution of 5% non-fat dry milk
in PBS to block the residual binding sites of the
membrane. The membrane was washed twice with PBS, and
then immersed in the blocking solution containing the
immune sera diluted to 1:1000. The membrane was
15 incubated with the antibody overnight at 4°C with gentle
shaking. The membrane was washed three times with PBS
and then incubated for 2 hours at room temperature with
alkaline phosphatase conjugated recombinant protein G
(1:1500 in PBS with 5% non-fat dry milk). The membrane
20 was washed three times with PBS and bound antibody was
detected by the addition of substrate (KPI BCIP/NBT
phosphatase substrate system; Kirkegaard and Perry,
Inc.). The immune sera reacted with six strains as
strongly, or to a greater extent than, strain O35E;
25 while the immune sera showed a slightly weaker
reactivity to three strains than strain O35E. Thus,
the antibodies elicited by immunization of CD protein
isolated from strain O35E cross-reacted with all
heterologous strains tested.

25 For vaccine development, CD specific amino acid
sequences may be purified from *B. catarrhalis* or may be
purified from a host containing a recombinant vector
which expresses CD or CD peptides. Such hosts include,
but are not limited to, bacterial transformants, yeast
30 transformants, filamentous fungal transformants, and
cultured cells that have been either infected or
transfected with a vector which encodes CD amino acid
sequences. Peptides or oligopeptides corresponding to
portions of the CD protein may be produced from chemical
35 or enzymatic cleavage of CD protein (See for example,
Embodiment D); or chemically synthesized using methods

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known in the art and with the amino acid sequence deduced from the nucleotide sequence of the gene encoding CD as a reference. Alternatively, CD peptides may be produced from a recombinant vector (See for
5 example, Embodiment A). The protein, peptide, or oligopeptide immunogen is included as the relevant immunogenic material in the vaccine formulation, and in therapeutically effective amounts, to induce an immune response. Many methods are known for the introduction of
10 a vaccine formulation into the human or animal to be vaccinated. These include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, ocular, intranasal, and oral administration. The vaccine may further comprise a
15 physiological carrier such as a solution, a polymer or liposomes; and an adjuvant, or a combination thereof.

Various adjuvants are used in conjunction with vaccine formulations. The adjuvants aid by modulating the immune response and in attaining a more durable and
20 higher level of immunity using smaller amounts of vaccine antigen or fewer doses than if the vaccine antigen were administered alone. Examples of adjuvants include incomplete Freund's adjuvant, Adjuvant 65 (containing peanut oil, mannide monooleate and aluminum monostearate), oil emulsions, Ribi adjuvant, the
25 pluronic polyols, polyamines, Avridine, Quil A, saponin, MPL, QS-21, and mineral gels such as aluminum hydroxide, aluminum phosphate, etc.

Another embodiment of this mode of the invention
30 involves the production of CD-specific amino acid sequences as a hapten, i.e. a molecule which cannot by itself elicit an immune response. In such case, the hapten may be covalently bound to a carrier or other immunogenic molecule which will confer immunogenicity to
35 the coupled hapten when exposed to the immune system.

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Thus, such a CD-specific hapten linked to a carrier molecule may be the immunogen in a vaccine formulation.

Another mode of this embodiment provides for either a live recombinant viral vaccine, recombinant bacterial vaccine, recombinant attenuated bacterial vaccine, or an inactivated recombinant viral vaccine which is used to protect against infections caused by *B. catarrhalis*. Vaccinia virus is the best known example, in the art, of an infectious virus that is engineered to express vaccine antigens derived from other organisms. The recombinant live vaccinia virus, which is attenuated or otherwise treated so that it does not cause disease by itself, is used to immunize the host. Subsequent replication of the recombinant virus within the host provides a continual stimulation of the immune system with the vaccine antigens such as CD protein, or CD peptides, thereby providing long-lasting immunity.

Other live vaccine vectors include: adenovirus, cytomegalovirus, and preferably the poxviruses such as vaccinia (Paoletti and Panicali, U.S. Patent No. 4,603,112) and attenuated *Salmonella* strains (Stocker et al., U.S. Patent Nos. 5,210,035; 4,837,151; and 4,735,801; and Curtiss et al., 1988, Vaccine 6:155-160). Live vaccines are particularly advantageous because they continually stimulate the immune system which can confer substantially long-lasting immunity. When the immune response is protective against subsequent *B. catarrhalis* infection, the live vaccine itself may be used in a preventative vaccine against *B. catarrhalis*.

To illustrate this mode of the embodiment, using molecular biological techniques such as those illustrated in Embodiment A, the gene encoding CD, or a gene fragment encoding one or more CD peptides may be inserted into the vaccinia virus genomic DNA at a site which allows for expression of CD epitopes but does not negatively affect the growth or replication of the

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vaccinia virus vector. The resultant recombinant virus can be used as the immunogen in a vaccine formulation. The same methods can be used to construct an inactivated recombinant viral vaccine formulation except that the recombinant virus is inactivated, such as by chemical means known in the art, prior to use as an immunogen and without substantially affecting the immunogenicity of the expressed immunogen. A mixture of inactivated viruses which express different epitopes may be used in the formulation of a multivalent inactivated vaccine. In either case, the inactivated recombinant vaccine or mixture of inactivated viruses may be formulated with a suitable adjuvant in order to enhance the immunological response to the vaccine antigens.

In another variation of this embodiment, genetic material is used directly as the vaccine formulation. Nucleic acid (DNA or RNA) containing sequences encoding CD protein, CD peptide or CD oligopeptide, operatively linked to one or more regulatory elements can be introduced directly to vaccinate the individual ("direct gene transfer") against pathogenic strains of *B. catarrhalis*. Direct gene transfer into a vaccinated individual, resulting in expression of the genetic material by the vaccinated individual's cells such as vascular endothelial cells as well as the tissue of the major organs, has been demonstrated by techniques in the art such as by injecting intravenously an expression plasmid:cationic liposome complex (Zhu et al., 1993, Science 261:209-211). Other effective methods for delivering vector DNA into a target cell are known in the art. In one example, purified recombinant plasmid DNA containing viral genes has been used to inoculate (whether parentally, mucosally, or via gene-gun immunization) vaccinees to induce a protective immune response (Fynan et al., 1993, Proc. Natl. Acad. Sci. USA 90:11478-11482). In another example, cells removed from

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an individual can be transfected or electroporated by standard procedures known in the art, resulting in the introduction of the recombinant vector DNA into the target cell. Cells containing the recombinant vector DNA may then be selected for using methods known in the art such as via a selection marker expressed in the vector, and the selected cells may then be re-introduced into the individual to express CD protein, CD peptide, or CD oligopeptide.

One preferred method of vaccination with genetic material comprises the step of administering to the individual the nucleic acid molecule that comprises a nucleic acid sequence that encodes for one or more of the CD protein, CD peptides, or CD oligopeptides, wherein the nucleic acid molecule is operatively linked to one or more regulatory sequences necessary for expression. The nucleic acid molecule can be administered directly, or first introduced into a viral vector and administered via the vector. The nucleic acid molecule can be administered in a pharmaceutically acceptable carrier or diluent and may contain compounds that can enhance the effectiveness of the vaccine. These additional compounds include, but are not limited to, adjuvants that enhance the immune response, and compounds that are directed to modulate the immune response, e.g. cytokines, collectively referred to as "immune modulators"; or other compounds which increase the uptake of nucleic acid by the cells, referred to as "nucleic acid uptake enhancers". The immunization with the nucleic acid molecule can be through any parental route (intravenous, intraperitoneal, intradermal, subcutaneous, or intramuscular), or via contact with mucosal surfaces of the nasopharynx, trachea, or gastrointestinal tract.

As an alternative to active immunization, such as where an immunocompromised individual is suffering from

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a potentially life-threatening infection caused by *B. catarrhalis*, immunization may be passive, i.e. immunization comprising administration of purified human immunoglobulin containing antibody against CD epitopes.

5

It should be understood that while the invention has been described in detail herein, the examples were for illustrative purposes only. Other modifications of the embodiments of the present invention that are
10 obvious to those skilled in the art of molecular biology, medical diagnostics, and related disciplines are intended to be within the scope of the appended claims.

15 What is claimed is:

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SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- (i) APPLICANT: Timothy F. Murphy
- (ii) TITLE OF INVENTION: Vaccine For *Branhamella catarrhalis*
- (iii) NUMBER OF SEQUENCES: 52
- 5 (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: Hodgson, Russ, Andrews, Woods & Goodyear
- (B) STREET: 1800 One M&T Plaza
- 10 (C) CITY: Buffalo
- (D) STATE: New York
- (E) COUNTRY: United States
- (F) ZIP: 14203-2391
- (v) COMPUTER READABLE FORM:
- 15 (A) MEDIUM TYPE: Diskette, 3.5 inch, 1.4 Mb storage
- (B) COMPUTER: IBM Compatible
- (C) OPERATING SYSTEM: MS-DOS/ Microsoft Windows 3.1
- (D) SOFTWARE: Wordperfect for Windows 5.1
- (vi) PRIOR APPLICATION DATA:
- 20 (A) APPLICATION NUMBER: U.S. 08/129,719
- (B) FILING DATE: September 29, 1993
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER: 08/306,871
- (B) FILING DATE: September 20, 1994
- 25 (vii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Nelson, M. Bud
- (B) REGISTRATION NUMBER: 35,300
- (C) REFERENCE DOCKET NUMBER: 11520.0053
- (viii) TELECOMMUNICATION INFORMATION:
- 30 (A) TELEPHONE: (716) 856-4000
- (B) TELEFAX: (716) 849-0349
- (2) INFORMATION FOR SEQ ID NO. 1:
- (i) SEQUENCE CHARACTERISTICS:
- 35 (A) LENGTH: 387 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double-stranded
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (iii) HYPOTHETICAL: yes
- 40 (iv) IMMEDIATE SOURCE:
- (A) LIBRARY: genomic
- (B) CLONE: lambda gt11 clone
- (v) ORIGINAL SOURCE:
- 45 (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240
- (C) CELL TYPE: bacterium
- (vi) FEATURE:
- 50 (A) LOCATION: CD gene region, 775-1160
- (B) IDENTIFICATION METHOD: by experiment
- (C) OTHER INFORMATION: contains sequence encoding epitopes recognized by CD-specific monoclonal antibodies 5E8 and 7D6

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(vii) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

5	GCT GGT TTA GAG GTA ACT TTG GGT GGT CGT TTG GCA GCT GCA	42
	Ala Gly Leu Glu Val Thr Leu Gly Gly Arg Leu Ala Pro Ala	
	1 5 10	
10	GTA CCA GTA GCA CCA GTG GCA GAA CCT GTT GCT GAA CCA GTT	84
	Val Pro Val Ala Pro Val Ala Glu Pro Val Ala Glu Pro Val	
	15 20 25	
15	GTT GCT CCA GCA CCT GTG ATC CTT CCT AAA CCA GAA CCT GAG	126
	Val Ala Pro Ala Pro Val Ile Leu Pro Lys Pro Glu Pro Glu	
	30 35 40	
20	CCT GTC ATT GAG GAA GCA CCA GCT GTA ATT GAA GAT ATT GTT	168
	Pro Val Ile Glu Glu Ala Pro Ala Val Ile Glu Asp Ile Val	
	45 50 55	
25	GTT GAT TCA GAC GGA GAT GGT GTG CCT GAT CAT CTG GAT GCC	210
	Val Asp Ser Asp Gly Asp Gly Val Pro Asp His Leu Asp Ala	
	60 65 70	
30	TGC CCA GGA ACT CCA GTA AAC ACT GTT GTT GAT CCA CGC GGT	252
	Cys Pro Glu Thr Pro Val Asn Thr Val Val Asp Pro Arg Gly	
	75 80	
35	TGC CCA GTA CAG GTT AAT TTG GTA GAA GAG CTT CGC CAA GAG	294
	Cys Pro Val Gln Val Asn Leu Val Glu Glu Leu Arg Gln Glu	
	85 90 95	
40	TTG CGT GTA TTC TTT GAT TAT GAT AAA TCA ATC ATC AAA CCA	336
	Leu Arg Val Phe Phe Asp Tyr Asp Lys Ser Ile Ile Lys Pro	
	100 105 110	
45	CAA TAC CGT GAA GAA GTT GCT AAG GTT GCT GCG CAA ATG CGT	378
	Gln Tyr Arg Glu Glu Val Ala Lys Val Ala Ala Gln Met Arg	
	115 120 125	
50	GAA TTC CCA	387
	Glu Phe Pro	
	129	

(3) INFORMATION FOR SEQ ID NO. 2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 20 nucleotides
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single-stranded
 - (D) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
- (A) ORGANISM: *Branhamella catarrhalis*
 - (B) STRAIN: 25240
- (iii) FEATURE:
- (A) LOCATION: CD gene region, 1116-1135
 - (B) IDENTIFICATION METHOD: by experiment

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- (C) OTHER INFORMATION: hybridizes to *Branhamella catarrhalis* gene region
- (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 2:
CCGTGAAGAA GTTGCTAAGG 20
- 5 (4) INFORMATION FOR SEQ ID NO. 3:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 15 nucleotides
- (B) TYPE: nucleic acid
- 10 (C) STRANDEDNESS: single-stranded
- (D) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
- (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240
- 15 (iii) FEATURE:
- (A) LOCATION: CD gene region, 206-220
- (B) IDENTIFICATION METHOD: by experiment
- (C) OTHER INFORMATION: hybridizes to *Branhamella catarrhalis* gene region
- 20 (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 3:
CTGACGAAGT CCACA 15
- (5) INFORMATION FOR SEQ ID NO. 4:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 16 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single-stranded
- (D) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
- 30 (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240
- (iii) FEATURE:
- (A) LOCATION: CD gene region (complementary strand),
316-331
- 35 (B) IDENTIFICATION METHOD: by experiment
- (C) OTHER INFORMATION: hybridizes to *Branhamella catarrhalis* gene region
- (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 4:
CACCAGTCCA TAGCTC 16
- 40 (6) INFORMATION FOR SEQ ID NO. 5:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 16 nucleotides
- (B) TYPE: nucleic acid
- 45 (C) STRANDEDNESS: single-stranded
- (D) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
- (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240
- 50 (iii) FEATURE:
- (A) LOCATION: CD gene region, 468-483
- (B) IDENTIFICATION METHOD: by experiment
- (C) OTHER INFORMATION: hybridizes to *Branhamella catarrhalis* gene region

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- (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 5:
GATTGGTACT GAGCAG 16
- (7) INFORMATION FOR SEQ ID NO. 6:
- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
- 10 (ii) ORIGINAL SOURCE:
(A) ORGANISM: *Branhamella catarrhalis*
(B) STRAIN: 25240
- (iii) FEATURE:
(A) LOCATION: CD gene region (complementary strand),
15 561-578
(B) IDENTIFICATION METHOD: by experiment
(C) OTHER INFORMATION: hybridizes to *Branhamella*
catarrhalis gene region
- (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 6:
20 GTATAACCAT CAATTGCA 18
- (8) INFORMATION FOR SEQ ID NO. 7:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 nucleotides
25 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
(A) ORGANISM: *Branhamella catarrhalis*
30 (B) STRAIN: 25240
- (iii) FEATURE:
(A) LOCATION: CD gene region, 724-738
(B) IDENTIFICATION METHOD: by experiment
(C) OTHER INFORMATION: hybridizes to *Branhamella*
35 *catarrhalis* gene region
- (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 7:
GCCCCGTGCTA TCCAT 15
- (9) INFORMATION FOR SEQ ID NO. 8:
- 40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
- 45 (ii) ORIGINAL SOURCE:
(A) ORGANISM: *Branhamella catarrhalis*
(B) STRAIN: 25240
- (iii) FEATURE:
(A) LOCATION: CD gene region (complementary strand),
50 826-842
(B) IDENTIFICATION METHOD: by experiment
(C) OTHER INFORMATION: hybridizes to *Branhamella*
catarrhalis gene region

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- (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 8:
GGTTCTGCCA CTGGTGC 17
- (10) INFORMATION FOR SEQ ID NO. 9:
- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
- 10 (ii) ORIGINAL SOURCE:
(A) ORGANISM: *Branhamella catarrhalis*
(B) STRAIN: 25240
- (iii) FEATURE:
(A) LOCATION: CD gene region (complementary strand),
15 1211-1225
(B) IDENTIFICATION METHOD: by experiment
(C) OTHER INFORMATION: hybridizes to *Branhamella*
catarrhalis gene region
- (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 9:
20 TGTAGCGTGC ACTTG 15
- (11) INFORMATION FOR SEQ ID NO. 10:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 nucleotides
25 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
(A) ORGANISM: *Branhamella catarrhalis*
30 (B) STRAIN: 25240
- (iii) FEATURE:
(A) LOCATION: CD gene region, 1387-1404
(B) IDENTIFICATION METHOD: by experiment
(C) OTHER INFORMATION: hybridizes to *Branhamella*
35 *catarrhalis* gene region
- (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 10:
GCAGTAATCA CTGGTAGC 18
- (12) INFORMATION FOR SEQ ID NO. 11:
- 40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
- 45 (ii) ORIGINAL SOURCE:
(A) ORGANISM: *Branhamella catarrhalis*
(B) STRAIN: 25240
- (iii) FEATURE:
(A) LOCATION: CD gene region (complementary strand),
50 1483-1497
(B) IDENTIFICATION METHOD: by experiment
(C) OTHER INFORMATION: hybridizes to *Branhamella*
catarrhalis gene region

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- (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 11:
TCGATAAGGC TTGAG 15
- (13) INFORMATION FOR SEQ ID NO. 12:
- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
- 10 (ii) ORIGINAL SOURCE:
(A) ORGANISM: *Branhamella catarrhalis*
(B) STRAIN: 25240
- (iii) FEATURE:
(A) LOCATION: CD gene region, 1665-1682
15 (B) IDENTIFICATION METHOD: by experiment
(C) OTHER INFORMATION: hybridizes to *Branhamella catarrhalis* gene region
- (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 12:
CGGGCATATC GCACGACT 18
- 20 (14) INFORMATION FOR SEQ ID NO. 13:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 nucleotides
(B) TYPE: nucleic acid
25 (C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
(A) ORGANISM: *Branhamella catarrhalis*
(B) STRAIN: 25240
- 30 (iii) FEATURE:
(A) LOCATION: CD gene region, 941-960
(B) IDENTIFICATION METHOD: by experiment
(C) OTHER INFORMATION: hybridizes to *Branhamella catarrhalis* gene region
- 35 (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 13:
TTGTTGATTC AGACGGAGAT 20
- (15) INFORMATION FOR SEQ ID NO. 14:
- (i) SEQUENCE CHARACTERISTICS:
40 (A) LENGTH: 1727 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- 45 (iii) HYPOTHETICAL: yes
- (iv) ORIGINAL SOURCE:
(A) ORGANISM: *Branhamella catarrhalis*
(B) STRAIN: 25240
- (v) IMMEDIATE SOURCE:
50 (A) LIBRARY: genomic
(B) CLONE: EMBL3 clone 5
(C) SUBCLONE: pCD1
- (vi) FEATURE:
(A) LOCATION: CD gene region

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- (B) IDENTIFICATION METHOD: by experiment
 (C) NAME/KEY: signal sequence of encoded protein
 (D) LOCATION: -26 to -1

(vii) SEQUENCE DESCRIPTION: SEQ ID NO. 14:

```

5  GGATCCCGTC GACCTGCAGG TCAACGGATC GCTATGCTAA AATAGGTGCG 50
   GTAATCTTGA AAAACCAACC ATTCCTTGGA GGAATTT  ATG AAA TTT 96
10                                     Met Lys Phe
                                       -26

   AAT AAA ATC GCT CTT GCG GTC ATC GCA GCC GTT GCA GCT CCA 138
   Asn Lys Ile Ala Leu Ala Val Ile Ala Ala Val Ala Ala Pro
                                       -15

15  GTT GCA GCT CCA GTT GCT GCT CAA GCT GGT GTG ACA GTC AGC 180
   Val Ala Ala Pro Val Ala Ala Gln Ala Gly Val Thr Val Ser
                                       -5          -1 1          5

20  CCA CTA CTA CTT GGC TAT CAT TAC ACT GAC GAA GCC CAC AAT 222
   Pro Leu Leu Leu Gly Tyr His Tyr Thr Asp Glu Ala His Asn
                                       10          15

25  GAT CAA CGC AAA ATC TTA CGC ACT GGC AAG AAG CTA GAG CTA 264
   Asp Gln Arg Lys Ile Leu Arg Thr Gly Lys Lys Leu Glu Leu
   20          25          30

30  GAT GCT ACT AAT GCA CCT GCA CCA GCT AAT GGC GGT GTC GCA 306
   Asp Ala Thr Asn Ala Pro Ala Pro Ala Asn Gly Gly Val Ala
   35          40          45

   CTG GAC AGT GAG CTA TGG ACT GGT GCT GCG ATT GGT ATC GAA 348
   Leu Asp Ser Glu Leu Trp Thr Gly Ala Ala Ile Gly Ile Glu
   50          55          60

35  CTT ACG CCA TCA ACT CAG TTC CAA GTT GAA TAT GGT ATC TCT 390
   Leu Thr Pro Ser Thr Gln Phe Gln Val Glu Tyr Gly Ile Ser
   65          70          75

40  AAC CGT GAT GCA AAA TCT TCA GAC AAA TCT GCA CAT CGC TTT 432
   Asn Arg Asp Ala Lys Ser Ser Asp Lys Ser Ala His Arg Phe
   80          85

45  GAT GCT GAG CAA GAA ACC ATC AGC GGT AAC TTT TTG ATT GGT 474
   Asp Ala Glu Gln Glu Thr Ile Ser Gly Asn Phe Leu Ile Gly
   90          95          100

   ACT GAG CAG TTC AGC GGC TAC AAT CCA ACA AAT AAA TTC AAG 516
   Thr Glu Gln Phe Ser Gly Tyr Asn Pro Thr Asn Lys Phe Lys
   105          110          115

50  CCC TAT GTC TTG GTT GGT GCA GGT CAA TCT AAA ATT AAA GTA 558
   Pro Tyr Val Leu Val Gly Ala Gly Gln Ser Lys Ile Lys Val
   120          125          130

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	AAT	GCA	ATT	GAT	GGT	TAT	ACA	GCA	GAA	GTA	GCC	AAT	GGG	CAA	600
	Asn	Ala	Ile	Asp	Gly	Tyr	Thr	Ala	Glu	Val	Ala	Asn	Gly	Gln	
				135					140					145	
5	AAC	ATT	GCA	AAA	GAT	CAA	GCT	GTA	AAA	GCA	GGT	CAA	GAA	GTT	642
	Asn	Ile	Ala	Lys	Asp	Gln	Ala	Val	Lys	Ala	Gly	Gln	Glu	Val	
				150					155						
10	GCT	GAG	TCT	AAA	GAC	ACC	ATC	GGT	AAC	CTA	GGT	CTT	GGT	GCT	684
	Ala	Glu	Ser	Lys	Asp	Thr	Ile	Gly	Asn	Leu	Gly	Leu	Gly	Ala	
	160				165					170					
15	CGC	TAC	TTA	GTC	AAT	GAT	GCC	CTT	GCA	CTT	CGT	GGT	GAA	GCC	726
	Arg	Tyr	Leu	Val	Asn	Asp	Ala	Leu	Ala	Leu	Arg	Gly	Glu	Ala	
	175				180						185				
20	CGT	GCT	ATC	CAT	AAT	TTT	GAT	AAC	AAA	TGG	TGG	GAA	GGC	TTG	768
	Arg	Ala	Ile	His	Asn	Phe	Asp	Asn	Lys	Trp	Trp	Glu	Gly	Leu	
			190					195					200		
	GCG	TTG	GCT	GGT	TTA	GAG	GTA	ACT	TTG	GGT	GGT	CGT	TTG	GCA	810
	Ala	Leu	Ala	Gly	Leu	Glu	Val	Thr	Leu	Gly	Gly	Arg	Leu	Ala	
				205					210					215	
25	CCT	GCA	GTA	CCA	GTA	GCA	CCA	GTG	GCA	GAA	CCT	GTT	GCT	GAA	852
	Pro	Ala	Val	Pro	Val	Ala	Pro	Val	Ala	Glu	Pro	Val	Ala	Glu	
				220					225						
30	CCA	GTT	GTT	GCT	CCA	GCA	CCT	GTG	ATC	CTT	CCT	AAA	CCA	GAA	894
	Pro	Val	Val	Ala	Pro	Ala	Pro	Val	Ile	Leu	Pro	Lys	Pro	Glu	
	230				235					240					
35	CCT	GAG	CCT	GTC	ATT	GAG	GAA	GCA	CCA	GCT	GTA	ATT	GAA	GAT	936
	Pro	Glu	Pro	Val	Ile	Glu	Glu	Ala	Pro	Ala	Val	Ile	Glu	Asp	
	245				250						255				
40	ATT	GTT	GTT	GAT	TCA	GAC	GGA	GAT	GGT	GTG	CCT	GAT	CAT	CTG	978
	Ile	Val	Val	Asp	Ser	Asp	Gly	Asp	Gly	Val	Pro	Asp	His	Leu	
			260					265					270		
	GAT	GCC	TGC	CCA	GGA	ACT	CCA	GTA	AAC	ACT	GTT	GTT	GAT	CCA	1020
	Asp	Ala	Cys	Pro	Glu	Thr	Pro	Val	Asn	Thr	Val	Val	Asp	Pro	
				275					280					285	
45	CGC	GGT	TGC	CCA	GTA	CAG	GTT	AAT	TTG	GTA	GAA	GAG	CTT	CGC	1062
	Arg	Gly	Cys	Pro	Val	Gln	Val	Asn	Leu	Val	Glu	Glu	Leu	Arg	
				290					295						
50	CAA	GAG	TTG	CGT	GTA	TTC	TTT	GAT	TAT	GAT	AAA	TCA	ATC	ATC	1104
	Gln	Glu	Leu	Arg	Val	Phe	Phe	Asp	Tyr	Asp	Lys	Ser	Ile	Ile	
	300				305						310				

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	AAA	CCA	CAA	TAC	CGT	GAA	GAA	GTT	GCT	AAG	GTT	GCT	GCG	CAA	1146
	Lys	Pro	Gln	Tyr	Arg	Glu	Glu	Val	Ala	Lys	Val	Ala	Ala	Gln	
		315					320					325			
5	ATG	CGT	GAA	TTC	CCA	AAT	GCA	ACT	GCA	ACC	ATT	GAA	GGT	CAC	1188
	Met	Arg	Glu	Phe	Pro	Asn	Ala	Thr	Ala	Thr	Ile	Glu	Gly	His	
			330					335					340		
10	GCA	TCA	CGC	GAT	TCA	GCA	CGC	TCA	AGT	GCA	CGC	TAC	AAC	CAG	1230
	Ala	Ser	Arg	Asp	Ser	Ala	Arg	Ser	Ser	Ala	Arg	Tyr	Asn	Gln	
				345					350					355	
15	CGT	CTA	TCT	GAA	GCT	CGT	GCT	AAT	GCT	GTT	AAA	TCA	ATG	CTA	1272
	Arg	Leu	Ser	Glu	Ala	Arg	Ala	Asn	Ala	Val	Lys	Ser	Met	Leu	
					360					365					
20	TCT	AAC	GAA	TTT	GGT	ATC	GCT	CCA	AAC	CGC	CTA	AAT	GCA	GTT	1314
	Ser	Asn	Glu	Phe	Gly	Ile	Ala	Pro	Asn	Arg	Leu	Asn	Ala	Val	
		370				375					380				
25	GGT	TAT	GGC	TTT	GAT	CGT	CCT	ATC	GCT	CCA	AAT	ACT	ACT	GCT	1356
	Gly	Tyr	Gly	Phe	Asp	Arg	Pro	Ile	Ala	Pro	Asn	Thr	Thr	Ala	
			385				390					395			
30	GAA	GGT	AAA	GCG	ATG	AAC	CGT	CGT	GTA	GAA	GCA	GTA	ATC	ACT	1398
	Glu	Gly	Lys	Ala	Met	Asn	Arg	Arg	Val	Glu	Ala	Val	Ile	Thr	
			400					405					410		
35	GGT	AGC	AAA	ACA	ACG	ACT	GTT	GAT	CAA	ACC	AAA	GAT	ATG	ATT	1440
	Gly	Ser	Lys	Thr	Thr	Thr	Val	Asp	Gln	Thr	Lys	Asp	Met	Ile	
				415					420					425	
40	GTT	CAA	TAATTGCACA	TGAGTATTTG	GTAATCAGCT	TGAATTCTCA									1486
	Val	Gln													
		427													
45	AGCCTTATCG	ATAAAAAAGC	CACCTTTTTTG	GTGGCTTTTTT	TATTTGGTGT										1536
	AAATTTTTTG	TTCAGTTAGA	CTGATTTATG	TTATAATAAG	CGGTTTTCTT										1586
	AGCTTTTGAA	TAAATCAGAT	GAGTTAAGCC	AACTGACTGA	TTTTATCAAT										1636
	TGAGTTATTT	TTAAGCCTTT	TATCAGTTTCG	GGCATATCGC	ACGACTATTA										1686
	ATCTTTATAT	GAGTATTTAT	GGCAGACGAC	ATTAAGCATT	T										1727

(16) INFORMATION FOR SEQ ID NO. 15:**(i) SEQUENCE CHARACTERISTICS:**

- 50 (A) LENGTH: 17 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- (A) ORGANISM: *Branhamella catarrhalis*

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- (B) STRAIN: 25240
- (iii) FEATURE:
- (A) LOCATION: CD gene region (complementary strand),
1430-1446
- 5 (B) IDENTIFICATION METHOD: by experiment
- (C) OTHER INFORMATION: hybridizes to *Branhamella*
catarrhalis gene region
- (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 15:
TTGAACAATC ATATCTT 17
- 10 (17) INFORMATION FOR SEQ ID NO. 16:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 18 nucleotides
- (B) TYPE: nucleic acid
- 15 (C) STRANDEDNESS: single-stranded
- (D) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
- (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240
- 20 (iii) FEATURE:
- (A) LOCATION: CD gene region, 166-183
- (B) IDENTIFICATION METHOD: by experiment
- (C) OTHER INFORMATION: hybridizes to *Branhamella*
catarrhalis gene region
- 25 (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 16:
GGTGTGACAG TCAGCCCA 18
- (18) INFORMATION FOR SEQ ID NO. 17:
- (i) SEQUENCE CHARACTERISTICS:
- 30 (A) LENGTH: 17 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single-stranded
- (D) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
- 35 (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240
- (iii) FEATURE:
- (A) LOCATION: CD gene region (complementary strand),
1081-1097
- 40 (B) IDENTIFICATION METHOD: by experiment
- (C) OTHER INFORMATION: hybridizes to *Branhamella*
catarrhalis gene region
- (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 17:
GATTATCAT AATCAAA 17
- 45 (19) INFORMATION FOR SEQ ID NO. 18:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 17 nucleotides
- (B) TYPE: nucleic acid
- 50 (C) STRANDEDNESS: single-stranded
- (D) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
- (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240

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- (iii) FEATURE:
 (A) LOCATION: CD gene region, 1048-1064.
 (B) IDENTIFICATION METHOD: by experiment
 (C) OTHER INFORMATION: hybridizes to *Branhamella*
catarrhalis gene region
- 5 (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 18:
 GTAGAAGAGC TTCGCCA 17
- (20) INFORMATION FOR SEQ ID NO. 19:
- 10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 105 residues
 (B) TYPE: amino acid
 (C) TOPOLOGY: linear
- 15 (ii) ORIGINAL SOURCE:
 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240
- (iii) FEATURE:
 (A) LOCATION: CD amino acid positions 1-105
- 20 (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 19:
- Gly Val Thr Val Ser Pro Leu Leu Leu Gly Tyr His Tyr Thr Asp
 1 5 10 15
- Glu Ala His Asn Asp Gln Arg Lys Ile Leu Arg Thr Gly Lys Lys
 20 25 30
- Leu Glu Leu Asp Ala Thr Asn Ala Pro Ala Pro Ala Asn Gly Gly
 35 40 45
- 30 Val Ala Leu Asp Ser Glu Leu Trp Thr Gly Ala Ala Ile Gly Ile
 50 55 60
- Glu Leu Thr Pro Ser Thr Gln Phe Gln Val Glu Tyr Gly Ile Ser
 65 70 75
- 35 Asn Arg Asp Ala Lys Ser Ser Asp Lys Ser Ala His Arg Phe Asp
 80 85 90
- 40 Ala Glu Gln Glu Thr Ile Ser Gly Asn Phe Leu Ile Gly Thr Glu
 95 100 105
- (21) INFORMATION FOR SEQ ID NO. 20:
- 45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear
- 50 (ii) ORIGINAL SOURCE:
 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240
- (iii) SEQUENCE DESCRIPTION: SEQ ID NO. 20:

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GCGCGCGGAT CCGGTGTGAC AGTCAGCCCA C 31

(22) INFORMATION FOR SEQ ID NO. 21:**(i) SEQUENCE CHARACTERISTICS:**

- 5 (A) LENGTH: 29 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- 10 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240

(iii) SEQUENCE DESCRIPTION: SEQ ID NO. 21:

ATATATGAAT TCCTCAGTAC CAATCAAAA 29

15

(23) INFORMATION FOR SEQ ID NO. 22:**(i) SEQUENCE CHARACTERISTICS:**

- 20 (A) LENGTH: 97 residues
 (B) TYPE: amino acid
 (C) TOPOLOGY: linear
 (ii) ORIGINAL SOURCE:
 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240

(iii) FEATURE:

- 25 (A) LOCATION: CD amino acid positions 106-202
 (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 22:

Gln Phe Ser Gly Tyr Asn Pro Thr Asn Lys Phe Lys Pro Tyr Val
 1 5 10 15

30

Leu Val Gly Ala Gly Gln Ser Lys Ile Lys Val Asn Ala Ile Asp
 20 25 30

35

Gly Tyr Thr Ala Glu Val Ala Asn Gly Gln Asn Ile Ala Lys Asp
 35 40 45

Gln Ala Val Lys Ala Gly Gln Glu Val Ala Glu Ser Lys Asp Thr
 50 55 60

40

Ile Gly Asn Leu Gly Leu Gly Ala Arg Tyr Leu Val Asn Asp Ala
 65 70 75

Leu Ala Leu Arg Gly Glu Ala Arg Ala Ile His Asn Phe Asp Asn
 80 85 90

45

Lys Trp Trp Glu Gly Leu Ala
 95

(24) INFORMATION FOR SEQ ID NO. 23:**(i) SEQUENCE CHARACTERISTICS:**

- 50 (A) LENGTH: 29 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear

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- (ii) ORIGINAL SOURCE:
 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240
- (iii) SEQUENCE DESCRIPTION: SEQ ID NO. 23:
 5 GCGCGCGGAT CCCAGTTCAG CGGCTACAA 29
- (25) INFORMATION FOR SEQ ID NO. 24:
 (i) SEQUENCE CHARACTERISTICS:
 10 (A) LENGTH: 30 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear
 (ii) ORIGINAL SOURCE:
 15 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240
 (iii) SEQUENCE DESCRIPTION: SEQ ID NO. 24:
 20 ATATATGAAT TCCGCCAAGC CTTCCCACCA 30
- (26) INFORMATION FOR SEQ ID NO. 25:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 59 residues
 (B) TYPE: amino acid
 25 (C) TOPOLOGY: linear
 (ii) ORIGINAL SOURCE:
 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240
 (iii) FEATURE:
 30 (A) LOCATION: CD amino acid positions 203-261
 (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 25:
 Leu Ala Gly Leu Glu Val Thr Leu Gly Gly Arg Leu Ala Pro Ala
 1 5 10 15
 35 Val Pro Val Ala Pro Val Ala Glu Pro Val Ala Glu Pro Val Val
 20 25 30
 40 Ala Pro Ala Pro Val Ile Leu Pro Lys Pro Glu Pro Glu Pro Val
 35 40 45
 Ile Glu Glu Ala Pro Ala Val Ile Glu Asp Ile Val Val Asp
 50 55 59
- (27) INFORMATION FOR SEQ ID NO. 26:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 nucleotides
 (B) TYPE: nucleic acid
 50 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear
 (ii) ORIGINAL SOURCE:
 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240
 (iii) SEQUENCE DESCRIPTION: SEQ ID NO. 26:

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GCGCGCGGAT CCTTGGCTGG TTTAGAGGTA A 31

(28) INFORMATION FOR SEQ ID NO. 27:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 32 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- 10 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240

(iii) SEQUENCE DESCRIPTION: SEQ ID NO. 27:

ATATATGAAT TCAACAACAA TATCTTCAAT TA 32

15

(29) INFORMATION FOR SEQ ID NO. 28:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 71 residues
 (B) TYPE: amino acid
 (C) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240

(iii) FEATURE:

- 25 (A) LOCATION: CD amino acid positions 261-331

(iv) SEQUENCE DESCRIPTION: SEQ ID NO. 28:

Asp Ser Asp Gly Asp Gly Val Pro Asp His Leu Asp Ala Cys Pro
 1 5 10 15

30

Glu Thr Pro Val Asn Thr Val Val Asp Pro Arg Gly Cys Pro Val
 20 25 30

Gln Val Asn Leu Val Glu Glu Leu Arg Gln Glu Leu Arg Val Phe
 35 35 40 45

Phe Asp Tyr Asp Lys Ser Ile Ile Lys Pro Gln Tyr Arg Glu Glu
 50 55 60

40 Val Ala Lys Val Ala Ala Gln Met Arg Glu Phe
 65 70

(30) INFORMATION FOR SEQ ID NO. 29:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 29 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- 50 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240

(iii) SEQUENCE DESCRIPTION: SEQ ID NO. 29:

GCGCGCGGAT CCGATTCAGA CGGAGATGG 29

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(31) INFORMATION FOR SEQ ID NO. 30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single-stranded
- (D) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240

(iii) SEQUENCE DESCRIPTION: SEQ ID NO. 30:

ATATATGAAT TCGAATTCAC GCATTTGCG 29

(32) INFORMATION FOR SEQ ID NO. 31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 residues
- (B) TYPE: amino acid
- (C) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240

(iii) FEATURE:

- (A) LOCATION: CD amino acid positions 261-301

(iv) SEQUENCE DESCRIPTION: SEQ ID NO. 31:

Asp Ser Asp Gly Asp Gly Val Pro Asp His Leu Asp Ala Cys Pro
 1 5 10 15

Glu Thr Pro Val Asn Thr Val Val Asp Pro Arg Gly Cys Pro Val
 20 25 30

Gln Val Asn Leu Val Glu Glu Leu Arg Gln Glu
 35 40

(33) INFORMATION FOR SEQ ID NO. 32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single-stranded
- (D) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240

(iii) SEQUENCE DESCRIPTION: SEQ ID NO. 32:

ATATATGAAT TCATCCAGAT GATCAGGCA 29

(34) INFORMATION FOR SEQ ID NO. 33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 residues
- (B) TYPE: amino acid
- (C) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- (A) ORGANISM: *Branhamella catarrhalis*

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(B) STRAIN: 25240

(iii) FEATURE:

(A) LOCATION: CD amino acid positions 286-311

(iv) SEQUENCE DESCRIPTION: SEQ ID NO. 33:

5 Arg Gly Cys Pro Val Gln Val Asn Leu Val Glu Glu Leu Arg Gln
 1 5 10 15

10 Glu Leu Arg Val Phe Phe Asp Tyr Asp Lys Ser
 20 25

(35) INFORMATION FOR SEQ ID NO. 34:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 29 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

20 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240

(iii) SEQUENCE DESCRIPTION: SEQ ID NO. 34:

GCGCGCGGAT CCCGCGGTTG CCCAGTACA 29

25 (36) INFORMATION FOR SEQ ID NO. 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

30 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240

(iii) SEQUENCE DESCRIPTION: SEQ ID NO. 35:

35 ATATATGAAT TCGATTTATC ATAATCAAA 29

(37) INFORMATION FOR SEQ ID NO. 36:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 16 residues
 (B) TYPE: amino acid
 (C) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

45 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240

(iii) FEATURE:

(A) LOCATION: CD amino acid positions 286-301

(iv) SEQUENCE DESCRIPTION: SEQ ID NO. 36:

50 Arg Gly Cys Pro Val Gln Val Asn Leu Val Glu Glu Leu Arg Gln
 1 5 10 15

Glu

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- (38) INFORMATION FOR SEQ ID NO. 37:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 30 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single-stranded
- (D) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
- (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240
- (iii) SEQUENCE DESCRIPTION: SEQ ID NO. 37:
- ATATATGAAT TCCTCTTGGC GAAGCTCTTC 30
- (39) INFORMATION FOR SEQ ID NO. 38:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 11 residues
- (B) TYPE: amino acid
- (C) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
- (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240
- (iii) FEATURE:
- (A) LOCATION: CD amino acid positions 293-303
- (iv) SEQUENCE DESCRIPTION: SEQ ID NO. 38:
- Asn Leu Val Glu Glu Leu Arg Gln Glu Leu Arg
- 1 5 10
- (40) INFORMATION FOR SEQ ID NO. 39:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 29 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single-stranded
- (D) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
- (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240
- (iii) SEQUENCE DESCRIPTION: SEQ ID NO. 39:
- GCGCGCGGAT CCAATTGCTT AGAAGAGCT 29
- (41) INFORMATION FOR SEQ ID NO. 40:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 29 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single-stranded
- (D) TOPOLOGY: linear
- (ii) ORIGINAL SOURCE:
- (A) ORGANISM: *Branhamella catarrhalis*
- (B) STRAIN: 25240
- (iii) SEQUENCE DESCRIPTION: SEQ ID NO. 40:
- ATATATGAAT TCACGCAACT CTTGGCGAA 29

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(42) INFORMATION FOR SEQ ID NO. 41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 residues
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- (A) ORGANISM: *Branhamella catarrhalis*
(B) STRAIN: 25240

(iii) FEATURE:

- (A) LOCATION: CD amino acid positions 295-311
(iv) SEQUENCE DESCRIPTION: SEQ ID NO. 41:

Val Glu Glu Leu Arg Gln Glu Leu Arg Val Phe Phe Asp Tyr Asp
1 5 10 15
Lys Ser
17

(43) INFORMATION FOR SEQ ID NO. 42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- (A) ORGANISM: *Branhamella catarrhalis*
(B) STRAIN: 25240

(iii) SEQUENCE DESCRIPTION: SEQ ID NO. 42:

GCGCGCGGAT CCGTAGAAGA GCTTCGCCA 29

(44) INFORMATION FOR SEQ ID NO. 43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single-stranded
(D) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- (A) ORGANISM: *Branhamella catarrhalis*
(B) STRAIN: 25240

(iii) SEQUENCE DESCRIPTION: SEQ ID NO. 43:

ATATATGAAT TCGATTTATC ATAATCAAA 29

(45) INFORMATION FOR SEQ ID NO. 44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 residues
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- (A) ORGANISM: *Branhamella catarrhalis*
(B) STRAIN: 25240

(iii) FEATURE:

(iv) (A) LOCATION: CD amino acid positions 311-331
SEQUENCE DESCRIPTION: SEQ ID NO. 44:

10 (46) INFORMATION FOR SEQ ID NO. 45:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 29 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single-stranded
 15 (D) TOPOLOGY: linear
 (ii) ORIGINAL SOURCE:
 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240
 (iii) SEQUENCE DESCRIPTION: SEQ ID NO. 45:

(47) INFORMATION FOR SEQ ID NO. 46:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 29 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single-stranded

(D) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

30 (A) ORGANISM: *Branhamella catarrhalis*

(B) STRAIN: 25240

(iii) SEQUENCE DESCRIPTION: SEQ ID NO. 46:

ATATATGAAT TCGAATTCAC GCATTGCG 29

(48) INFORMATION FOR SEQ ID NO. 47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 59 residues

(B) TYPE: amino acid

(C) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

(A) ORGANISM: *Branhamella catarrhalis*

(B) STRAIN: 25240

(iii) FEATURE:

(A) LOCATION: CD amino acid positions 332-390

(iv) SEQUENCE DESCRIPTION: SEQ ID NO. 47:

Pro Asn Ala Thr Ala Thr Ile Glu Gly His Ala Ser Arg Asp Ser
1 5 10 15

Ala Arg Ser Ser Ala Arg Tyr Asn Gln Arg Leu Ser Glu Ala Arg
 20 25 30

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Ala Asn Ala Val Lys Ser Met Leu Ser Asn Glu Phe Gly Ile Ala
 35 40 45

5 Pro Asn Arg Leu Asn Ala Val Gly Tyr Gly Phe Asp Arg Pro
 50 55 59

(49) INFORMATION FOR SEQ ID NO. 48:**(i) SEQUENCE CHARACTERISTICS:**

- 10 (A) LENGTH: 29 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- 15 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240

(iii) SEQUENCE DESCRIPTION: SEQ ID NO. 48:

GCGCGCGGAT CCCCAAATGC AACTGCAAC 29

(50) INFORMATION FOR SEQ ID NO. 49:**(i) SEQUENCE CHARACTERISTICS:**

- 20 (A) LENGTH: 29 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- 25 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240

(iii) SEQUENCE DESCRIPTION: SEQ ID NO. 49:

30 ATATATGAAT CCAGGACGAT CAAAGCCAT 29

(51) INFORMATION FOR SEQ ID NO. 50:**(i) SEQUENCE CHARACTERISTICS:**

- 35 (A) LENGTH: 37 residues
 (B) TYPE: amino acid
 (C) TOPOLOGY: linear

(ii) ORIGINAL SOURCE:

- 40 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240

(iii) FEATURE:

- (A) LOCATION: CD amino acid positions 391-427

(iv) SEQUENCE DESCRIPTION: SEQ ID NO. 50:

45 Ile Ala Pro Asn Thr Thr Ala Glu Gly Lys Ala Met Asn Arg Arg
 1 5 10 15

Val Glu Ala Val Ile Thr Gly Ser Lys Thr Thr Thr Val Asp Gln
 20 25 30

50 Thr Lys Asp Met Ile Val Gln
 35 37

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(52) INFORMATION FOR SEQ ID NO. 51:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 29 nucleotides
 (B) TYPE: nucleic acid
5 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear
 (ii) ORIGINAL SOURCE:
 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240
10 (iii) SEQUENCE DESCRIPTION: SEQ ID NO. 51:

GCGCGCGGAT CCATCGCTCC AAATACTAC 29

(53) INFORMATION FOR SEQ ID NO. 52:

- 15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 33 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single-stranded
 (D) TOPOLOGY: linear
20 (ii) ORIGINAL SOURCE:
 (A) ORGANISM: *Branhamella catarrhalis*
 (B) STRAIN: 25240
 (iii) SEQUENCE DESCRIPTION: SEQ ID NO. 52:

25 ATATATGAAT TCTTGAACAA TCATATCTTT GGT 33

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1. A vaccine formulation comprising an immunologically effective amount of a substantially pure peptide, oligopeptide, or protein having one or more epitopes of CD, wherein CD is an outer membrane protein of
5 *Branhamella catarrhalis* of an apparent molecular mass of from about 55,000 to about 60,000 daltons and which is encoded by a nucleotide sequence shown as an open reading frame in SEQ ID NO. 14.
- 10 2. The vaccine formulation according to claim 1, wherein the protein having one or more epitopes of CD consists of an amino acid sequence substantially as depicted in SEQ IN NO. 14 from amino acid residue 1 to 427.
- 15 3. The vaccine formulation according to claim 1, wherein the protein having one or more epitopes of CD consists of an amino acid sequence substantially as depicted in SEQ IN NO. 14 from amino acid residue -26 to
20 427.
- 25 4. The vaccine formulation according to claim 1, in which the peptide, oligopeptide or protein was produced recombinantly from cells cultured from a host cell system genetically engineered to include a vector containing a nucleotide sequence that regulates expression of DNA sequences encoding CD epitopes, said host cell system is selected from the group consisting of bacteria, yeast, filamentous fungi, insect cell
30 lines, and mammalian cell lines.
5. The vaccine formulation according to claim 1, in which the peptide or oligopeptide is produced by chemical or enzymatic cleavage of CD protein.

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6. The vaccine formulation according to claim 1, in which the peptide or oligopeptide is produced by chemical synthesis.
- 5 7. The vaccine formulation according to claim 1, further comprising a pharmaceutically acceptable carrier or diluent.
8. The vaccine formulation according to claim 7,
10 further comprising an immune modulator.
9. The vaccine formulation of claim 1, wherein the peptide, or oligopeptide having one or more CD epitopes is selected from the group consisting of SEQ ID NO. 1,
15 SEQ ID NO. 19, SEQ ID NO. 22, SEQ ID NO. 25, SEQ ID NO. 28, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 41, SEQ ID NO. 44, SEQ ID NO. 47, and SEQ ID NO. 50.
- 20 10. The vaccine formulation according to claim 4, wherein the nucleotide sequence encoding CD epitopes is selected from the group consisting of SEQ ID NO. 1 and SEQ ID NO. 14.
- 25 11. The vaccine formulation according to claim 4, in which the cultured cell is a bacterium.
12. The vaccine formulation according to claim 4, in which the cultured cell is a yeast.
30
13. The vaccine formulation according to claim 4, in which the cultured cell is a filamentous fungus.
14. The vaccine formulation according to claim 4, in
35 which the cultured cell is an insect cell line.

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15. The vaccine formulation according to claim 4, in which the cultured cell is a mammalian cell line.

16. A substantially pure antigenic peptide,
5 oligopeptide, or protein having one or more epitopes of CD, wherein CD is an outer membrane protein of *Branhamella catarrhalis* of an apparent molecular mass of from about 55,000 to about 60,000 daltons and having an amino acid sequence substantially as depicted in SEQ ID
10 NO. 14.

17. The protein according to claim 16, wherein the protein having one or more epitopes of CD consists of an amino acid sequence substantially as depicted in SEQ IN
15 NO. 14 from amino acid residue 1 to 427.

18. The protein according to claim 16, wherein the protein having one or more epitopes of CD consists of an amino acid sequence substantially as depicted in SEQ IN
20 NO. 14 from amino acid residue -26 to 427.

19. The peptide or oligopeptide according to claim 16, wherein the peptide or oligopeptide having one or more CD epitopes is selected from the group consisting of SEQ
25 ID NO. 1, SEQ ID NO. 19, SEQ ID NO. 22, SEQ ID NO. 25, SEQ ID NO. 28, SEQ ID NO. 31, SEQ ID NO. 33, SEQ ID NO. 36, SEQ ID NO. 38, SEQ ID NO. 41, SEQ ID NO. 44, SEQ ID NO. 47, and SEQ ID NO. 50.

20. The peptide, oligopeptide or protein according to claim 16, in which the peptide, oligopeptide, or protein was produced recombinantly from cells cultured from a host cell system genetically engineered to include a vector containing a nucleotide sequence that regulates
35 expression of DNA sequences encoding CD epitopes, said host cell system is selected from the group consisting

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of bacteria, yeast, filamentous fungi, insect cell lines, and mammalian cell lines.

21. The peptide, oligopeptide or protein according to
5 claim 20, in which the cultured cell is a bacterium.

22. The peptide, oligopeptide or protein according to
claim 20, in which the cultured cell is a yeast.

10 23. The peptide, oligopeptide or protein according to
claim 20, in which the cultured cell is a filamentous
fungus.

15 24. The peptide, oligopeptide or protein according to
claim 20, in which the cultured cell is an insect cell
line.

25 25. The peptide, oligopeptide or protein according to
claim 20, in which the cultured cell is a mammalian cell
20 line.

26. A recombinant vector comprising a DNA sequence
encoding one or more antigenic determinants or epitopes
of CD, wherein CD is an outer membrane protein of
25 *Branhamella catarrhalis* of an apparent molecular mass of
from about 55,000 to about 60,000 daltons and having an
amino acid sequence substantially as depicted in SEQ ID
NO. 14.

30 27. The recombinant vector according to claim 26,
wherein CD consists of an amino acid sequence
substantially as depicted in SEQ IN NO. 14 from amino
acid residue 1 to 427.

35 28. The recombinant vector according to claim 26,
wherein the protein having one or more epitopes of CD

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consists of an amino acid sequence substantially as depicted in SEQ IN NO. 14 from amino acid residue -26 to 427.

- 5 29. The recombinant vector of claim 26, wherein the vector is selected from the group consisting of a plasmid vector, phagemid vector, cosmid vector, and a viral vector.
- 10 30. A composition useful to passively immunize individuals suffering from an infection caused by *B. catarrhalis*, said composition comprising purified antiserum which recognizes one or more epitopes of CD, wherein CD is an outer membrane protein of *B.*
- 15 *catarrhalis* of an apparent molecular mass of from about 55,000 to about 60,000 daltons and having an amino acid sequence substantially as depicted in SEQ ID NO. 14 from amino acid residue 1 to 427.
- 20 31. An isolated gene or fragments thereof encoding epitopes of outer membrane protein CD of *Branhamella catarrhalis* having an apparent molecular mass of from about 55,000 to about 60,000 daltons, wherein said gene comprises the 1359 base pair open
- 25 reading frame of SEQ ID NO. 14.
- 30 32. A vaccine formulation comprising a nucleic acid molecule which encodes the CD protein, or one or more gene fragments that encodes one or more CD peptides or CD oligopeptides, said nucleic acid molecule is operatively linked with regulatory sequences.
- 35 33. The vaccine formulation according to claim 32, further comprising a pharmaceutically acceptable carrier or diluent.

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34. The vaccine formulation according to claim 33, further comprising an immune modulator.

5 35. The vaccine formulation according to claim 33, further comprising a nucleic acid uptake enhancer.

10 36. An infectious, recombinant microorganism capable of expressing CD protein, CD peptides or CD oligopeptides, of *B. catarrhalis*.

37. A microorganism of claim 36, which is a vaccinia virus, adenovirus, or cytomegalovirus.

15 38. A microorganism of claim 36, which is a bacterium of the genus *Salmonella*.

20 39. A method for the detection of *B. catarrhalis*-specific antisera in a body fluid of an individual comprising using peptide or protein having one or more epitopes of CD as an antigen in an immunoassay to interact with and detect *B. catarrhalis*-specific antisera in the body fluid, wherein CD is an outer membrane protein of *Branhamella catarrhalis* of an apparent molecular mass of from about 55,000 to about 25 60,000 daltons.

30 40. The method of claim 39, wherein the immunoassay is an assay selected from the group consisting of a radioimmunoassay, enzyme-linked immunosorbent assay, "sandwich" assay, precipitin reaction, agglutination assay, fluorescent-based immunoassay, and chemiluminescence-based immunoassay.

35 41. Oligonucleotides useful in the detection of *B. catarrhalis*, said oligonucleotides consisting essentially of nucleic acid sequences which complement

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and specifically hybridize to conserved regions of the gene contained within the nucleotide sequence of SEQ ID NO. 14 or its corresponding complementary strand.

5 42. The oligonucleotides of claim 41, wherein the oligonucleotides consist essentially of nucleic acid sequences selected from the group consisting of SEQ ID NO. 2; SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID
10 NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, SEQ ID NO. 13, SEQ ID NO. 15, SEQ ID NO. 16, SEQ ID NO. 17, or SEQ ID NO. 18.

15 43. A method for detecting the presence or absence of *Branhamella catarrhalis* in a clinical specimen, wherein the method comprises the steps of:

 (a) lysing the cells in the specimen to release bacterial genetic material;

20 (b) contacting the genetic material with two oligonucleotides under suitable conditions permitting hybridization of the oligonucleotides to the genetic material, wherein one oligonucleotide hybridizes to a region of a gene contained within the nucleotide sequence of SEQ ID NO. 14, and the other oligonucleotide
25 hybridizes to a region in the corresponding complementary strand;

 (c) enzymatically amplifying a specific region of sequence of the genetic material comprising the gene and its corresponding strand using the oligonucleotides of
30 step (b) as primers; and

 (d) detecting the presence of amplified sequences of the gene and its corresponding strand, wherein the presence of these amplified sequences correlate to the presence of *B. catarrhalis* in the specimen.

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44. The method of claim 43, wherein the detection is further facilitated by hybridization of amplified sequences with a labeled oligonucleotide probe consisting of a nucleotide sequence corresponding to a region in the specific region of sequence to be amplified, said label is a label known to be incorporated in oligonucleotides selected particularly from among radioactive label, such as ^{32}P , and enzymatic label, such as biotin.

10

45. The method of claim 43, wherein the specimen is a body fluid selected from the group consisting of middle ear fluid; sputum; blood; and fluids from the nasopharynx, or eye, or adenoid.

15

46. A method for the detection of *Branhamella catarrhalis* in a clinical specimen, wherein the method comprises the steps of:

- (a) obtaining a specimen of body fluid;
- 20 (b) lysing the cells in the specimen to release bacterial genetic material;
- (c) contacting the genetic material with an oligonucleotide probe synthesized to correspond to a region of a gene contained within the nucleotide sequence of SEQ ID NO. 14, or its corresponding complementary strand under suitable conditions permitting hybridization of the oligonucleotide to the genetic material; and
- 25 (d) detecting interaction between the specimen and the probe, said interaction being between the genetic material of *B. catarrhalis* and the probe.

30

47. The method of claim 46, wherein the specimen is a body fluid selected from the group consisting of middle ear fluid; sputum; blood; and fluids from the nasopharynx, or eye, or adenoid.

35

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FIG. 1

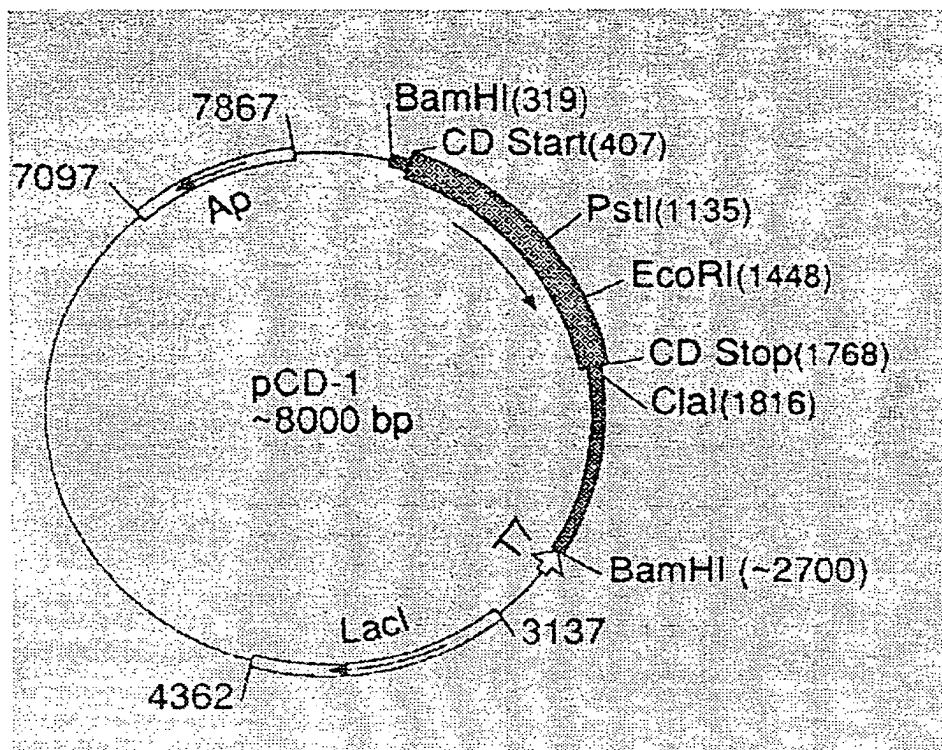
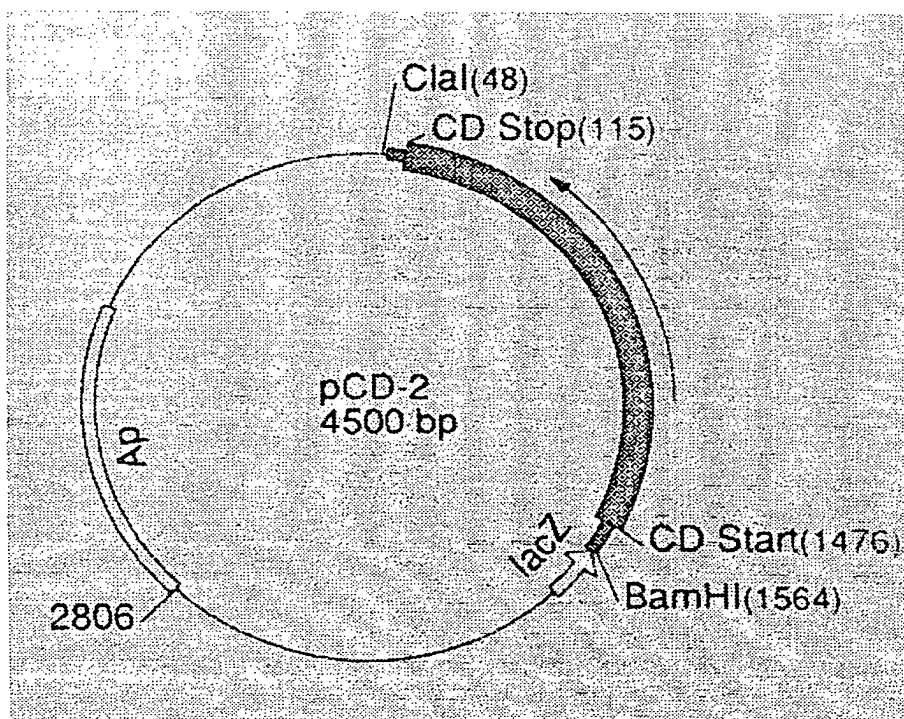


FIG. 2



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FIG. 3

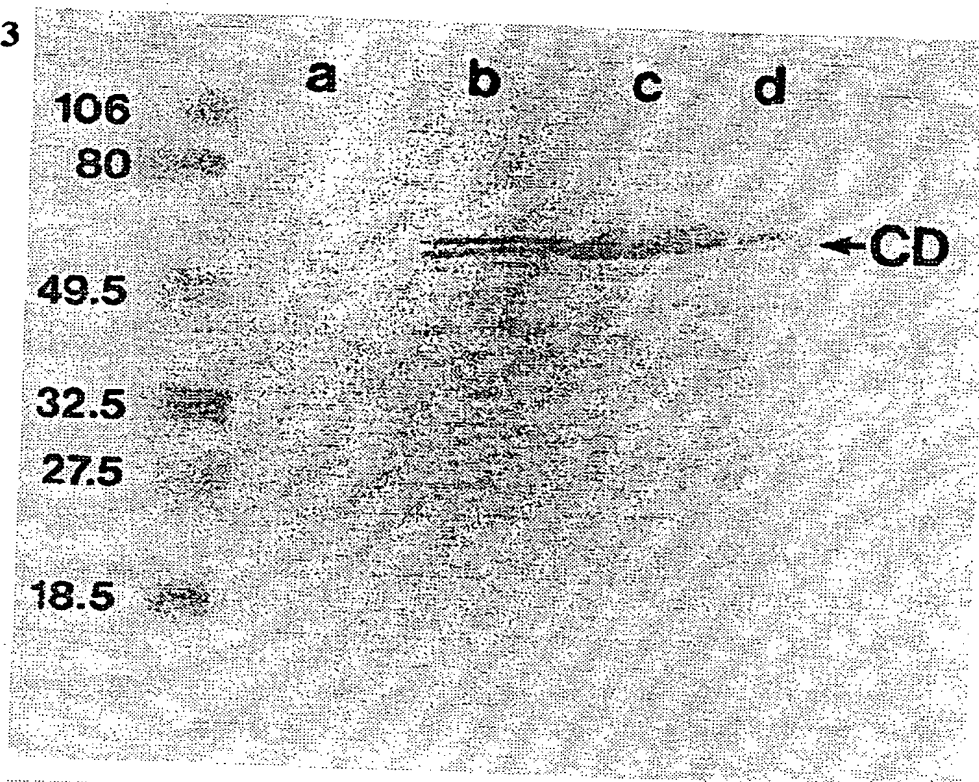
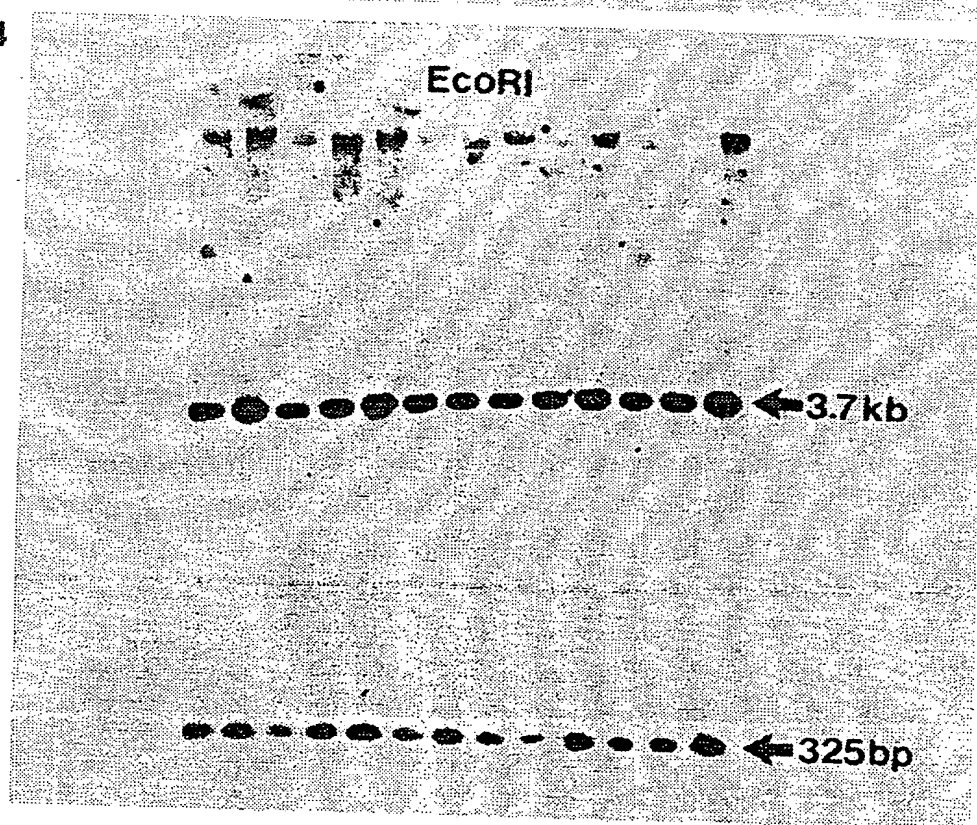
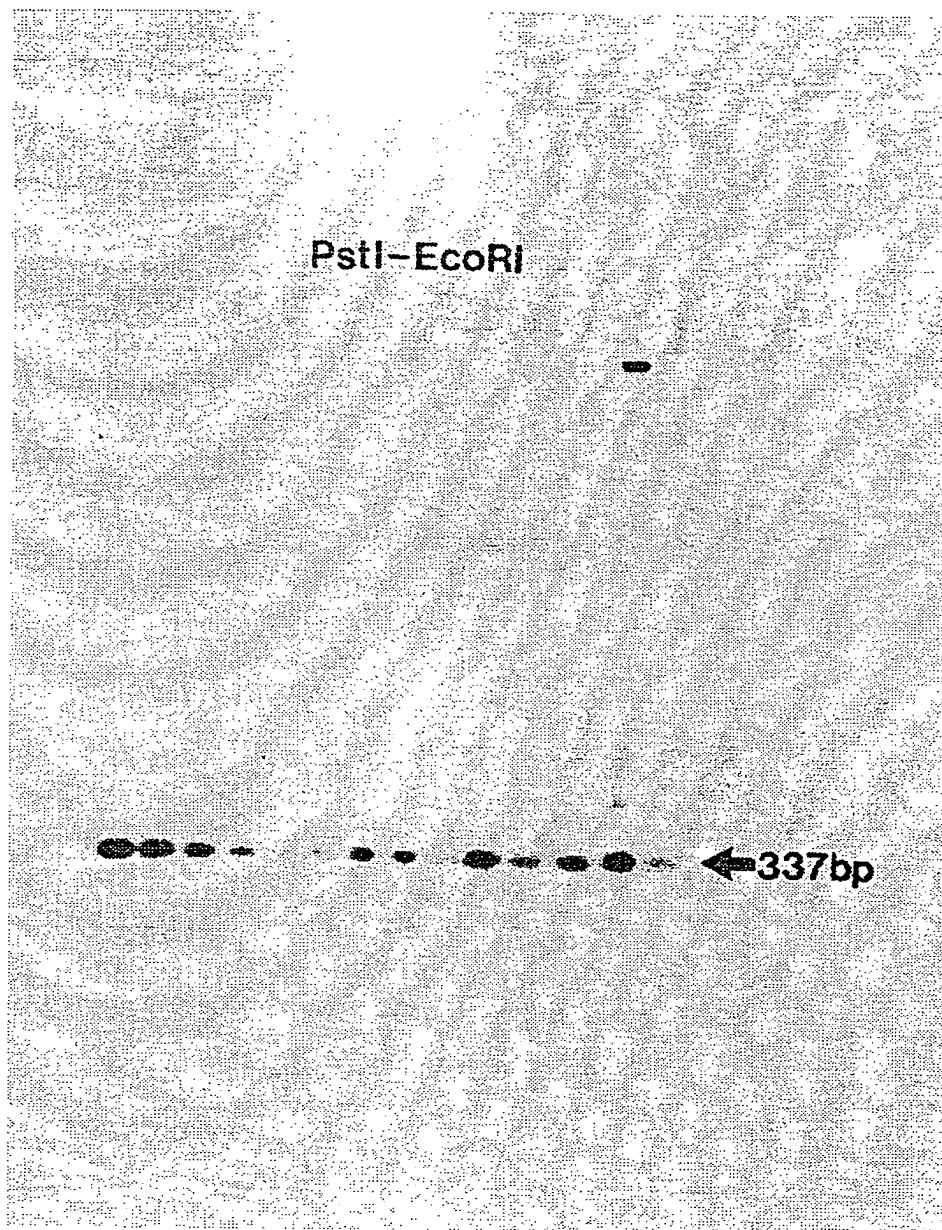


FIG. 4

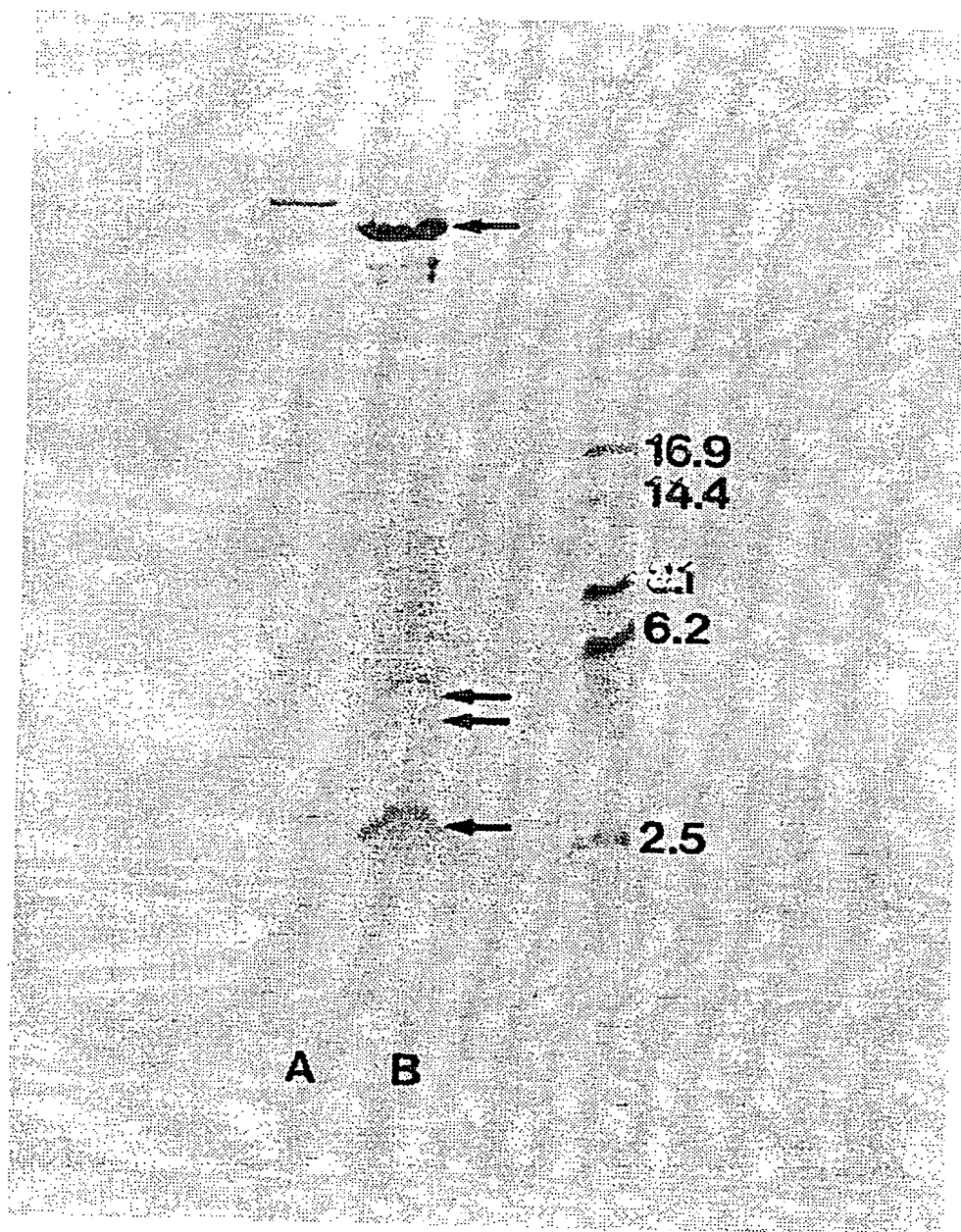


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FIG. 5



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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10932

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.57, 1.49; 530/324; 435/6, 7.32, 91.2, 320.1; 536/23.7, 24.32

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	INFECTION AND IMMUNITY, Volume 60, No. 3, issued March 1992, Sarwar et al., "Characterization of an Antigenically Conserved Heat-Modifiable major Outer Membrane Protein of <i>Branhamella catarrhalis</i> ", pages 804-809; see entire document.	1-40
Y	THE JOURNAL OF INFECTIOUS DISEASES, Volume 162, issued November 1990, Goldblatt et al., " <i>Branhamella catarrhalis</i> : Antigenic Determinants and the Development of the IgG Subclass Response in Childhood", pages 1128-1135; see the entire document.	1-40

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A document defining the general state of the art which is not considered to be of particular relevance	*X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E earlier document published on or after the international filing date	*Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*& document member of the same patent family
*O document referring to an oral disclosure, use, exhibition or other means	
*P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

05 DECEMBER 1994

Date of mailing of the international search report

18 JAN 1995

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/10932

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	INFECTION AND IMMUNITY, Volume 61, No. 5, issued May 1993, Helminen et al., "A Major Outer Membrane Protein of <i>Moraxella catarrhalis</i> Is a Target for Antibodies That Enhance Pulmonary Clearance of the Pathogen in an Animal Model", pages 2003-2010; see the entire document.	1-40
Y	INFECTION AND IMMUNITY, Volume 57, No. 10, issued October 1989, Murphy et al., "Surface-Exposed and Antigenically Conserved Determinants of Outer Membrane Proteins of <i>Branhamella catarrhalis</i> ", pages 2938-2941; see the entire document.	1-40
Y	US, A, 5,124,246 (URDEA ET AL.) 23 June 1992, see entire document.	41-47
Y, P	US, A, 5,292,874, (MILLIMAN) 08 March 1994, see entire document.	41-47
Y, P	US, A, 5,334,379 (PILLAI ET AL.) 02 August 1994, see entire document.	1-30
Y	US, A, 4,358,535 (FALKOW ET AL.) 09 November 1982, see entire document.	41-47
A	US, A, 5,256,536 (MIYADA ET AL.) 26 October 1993, see entire document.	41-47
A	US, A, 5,216,143 (HOGAN ET AL.) 01 June 1993, see entire document.	41, 42
A	US, A, 5,010,015 (PALVA) 23 April 1991, see entire document.	16-29, 31
Y	US, A, 4,683,195 (MULLIS ET AL.) 28 July 1987, see entire document.	43-45

Form PCT/ISA/210 (continuation of second sheet)(July 1992)★

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A61M 36/14; A61K 37/02; C12N 15/00; C12P 21/04; C12Q 1/00, 1/68; C07H 17/00; C12P 19/34

CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/1.57, 1.49; 530/324; 435/6, 7.32, 91.2, 320.1; 536/23.7, 24.32

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG

Search Terms: Branhamella catarrhalis, vaccine, outer membrane protein, CD, amplifi?, nucleic acid, DNA, polymerase chain reaction, PCR, peptide, oligonucleotide, primer, probe, immunoassay or ELISA, antibod?

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

I. Claims 1-15 and 32-35, drawn to a vaccine.

II. Claims 16-25, drawn to an antigenic peptide.

III. Claims 26-29, drawn to a recombinant vector.

IV. Claim 30, drawn to an immunoglobulin composition for use in passive immunity.

V. Claim 31, drawn to an isolated gene.

VI. Claims 36-38, drawn to an infectious microorganism.

VII. Claims 39-40, drawn to an immunoassay for the detection of Branhamella catarrhalis-specific antisera in a body fluid.

VIII. Claims 41 and 42, drawn to oligonucleotides; claims 43-45, drawn to a method of detecting Branhamella catarrhalis nucleic acid involving amplification reactions; and claims 46-47, drawn to a hybridization method of detecting nucleic acid from B. catarrhalis.

The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: While the inventions are all related in that they either are the peptide, a composition containing same, produce said peptides, or require its use (the vaccine of Group I is comprised of the peptides claimed in Group II; the antigenic peptide of Group II; the recombinant vector of Group III which encodes the peptide of Group II which is used in the vaccine of Group I; the immunoglobulins of Group IV which bind to the peptide of Group II; the isolated gene of Group V which encodes the peptide of Group II; the composition for passive immunity of Group IV which is comprised of immunoglobulins which bind to the peptides set forth in Group II; the isolated gene of group V which encodes the peptides of Group II; the infectious microorganism of Group VI which expresses the peptide of Group II; the immunoassay which tests for the presence of antibodies in a body fluid which bind to said peptides; and the oligonucleotides which hybridize to the nucleic acid that encodes the peptides and are used in the methods of detecting said nucleic acid as set forth in Group VIII) the inventions are not so linked by a special technical feature as the peptides as presently claimed were known in the prior art and therefore do not qualify as a special technical feature within the meaning of PCT Rule 13.2. In support of this position, attention is directed to the publication of Sarwar et al, Infection and Immunity, (1992) where in the abstract there is taught the expression of epitopes found in an outer membrane protein (OMP) of Branhamella catarrhalis which has a molecular mass of 55 kDa at room temperature and a mass of 60 kDa when heated under reducing conditions. At page 806, left column, there is taught the development of monoclonal antibodies which are specific for the protein OMP C/D. In comparison, it is noted that the claimed peptide and its various formulations and

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uses are derived from the outer membrane protein CD of *Branhamella catarrhalis*, which has a molecular weight of from about 55,000 to 60,000. While the publication of Sarwar et alii, does not teach the amino acid residue sequence, the two proteins are, in the absence of convincing evidence to the contrary, considered to be one and the same. Additionally, attention is directed to the publication of Goldblatt et al, *The Journal of Infectious Diseases* (1990) which teaches at page 1129 of the development of a ELISA based upon the use of OMPs of *Branhamella catarrhalis*. Again, in the absence of convincing evidence to the contrary, the OMPs used by Goldblatt et al, are considered to encompass that being claimed instantly. Therefore, as indicated above, the peptide which links the various Groups, is not a special technical feature. Thus, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.

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